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<b>(21) International Application Number:</b> PCT/US99/11869 <b>(22) International Filing Date:</b> 28 May 1999 (28.05.99)  <b>(30) Priority Data:</b> 60/087,274 29 May 1998 (29.05.98) US  <b>(71) Applicant (for all designated States except US):</b> THE GOVERNMENT OF THE UNITED STATES as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; c/o National Institutes of Health, 6011 Executive Boulevard, Box 13, Rockville, MD 20852-3804 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> CLARK, Janet [US/US]; 7530 Weatherby Drive, Rockville, MD 20855 (US). BONNER, Thomas, I. [US/US]; 6918 Maple Avenue, Chevy Chase, MD 20815 (US).  <b>(74) Agents:</b> SAMPLES, Kenneth, H. et al.; Fitch, Even, Tabin & Flannery, Suite 1600, 120 S. LaSalle Street, Chicago, IL 60603-3406 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> MAMMALIAN gb2 GABA <sub>B</sub> RECEPTORS		
<b>(57) Abstract</b>		
<p>The present invention provides a mammalian GABA<sub>B</sub> receptor subunit polypeptide, namely the gb2 subunit, as well as a DNA encoding the subunit. The gb2 subunit, when in conjunction with a mammalian gb1 polypeptide, expresses GABA<sub>B</sub> receptor activity. Vectors for expressing the gb2 polypeptide and cells expressing it are part of the invention, as are methods of producing a gb2 GABA<sub>B</sub> receptor subunit in a mammalian cell, determining the ability of a substance to bind to a mammalian GABA<sub>B</sub> receptor, and evaluating whether a substance modulates an expressed activity of a GABA<sub>B</sub> receptor. The invention also discloses an antibody that reacts immunospecifically with a mammalian gb2 receptor subunit.</p>		

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Exhibit 7

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## MAMMALIAN $\text{gb2 GABA}_B$ RECEPTORS

### Field of The Invention

5 The present invention relates to a new subtype of mammalian  $\text{GABA}_B$  receptor. This subtype has been designated  $\text{gb2 GABA}_B$ . In particular, the present invention relates to nucleic acids (DNA) encoding  $\text{gb2 GABA}_B$ , to vectors and host expression systems containing these nucleic acids, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and to antibodies produced against  
10 these antigens.

### Background

GABA ( $\gamma$ -aminobutyric acid or 4-aminobutanoic acid) is the major inhibitory neurotransmitter in the mammalian brain. GABAergic inhibitory synaptic  
15 transmission is mediated by two distinct types of receptors, ligand-gated chloride channels and G-protein-coupled receptors. The multi-subunit  $\text{GABA}_A$  receptors constitute the ligand-gated channels that when activated allow the passage of chloride ions into the cell resulting in hyperpolarization and cessation of neurotransmission.  $\text{GABA}_B$  receptors, which are abundantly and heterogeneously distributed throughout  
20 the central nervous system, are members of the G-protein-coupled receptor superfamily and mediate a variety of inhibitory cellular processes through pertussis toxin-sensitive G-proteins  $G_o$  and/or  $G_i$ . The G-proteins (i.e., guanine nucleotide binding proteins) allow functional coupling of the  $\text{GABA}_B$  receptors to  $\text{Ca}^{++}$  and  $\text{K}^+$  channels. Activation of  $\text{GABA}_B$  receptors has been shown to reduce evoked  
25 neurotransmitter release through modulation of voltage-sensitive  $\text{Ca}^{++}$  and/or  $\text{K}^+$  currents, hyperpolarize a postsynaptic cell through activation of  $\text{K}^+$  currents, and regulate adenylyl cyclase activity (see, for example, review articles by Bowery, Annu. Rev. Pharmacol. Toxicol. 33, 109-147 (1993); Kerr et al., Pharmac. Ther. 67, 187-246 (1995); Misgeld et al., Prog. Neurobiol. 46, 423-462 (1995)).  $\text{GABA}_B$  receptors were  
30 originally identified due to their insensitivity to the  $\text{GABA}_A$  antagonist bicuculline and their sensitivity to the agonist baclofen (Bowery et al., Eur. J. Pharmacol. 71, 53-70 (1981); Hill et al., Nature 290, 149-152 (1981)).

Second messenger and electrophysiological studies of pre- and postsynaptic GABA<sub>B</sub> receptors suggest that there may be as many as four receptor subtypes (Gemignani et al., Mol. Pharmacol. 46, 558-562 (1994); Cunningham et al., Brain Res. 720, 220-224 (1996); Bonanno et al., Trends Pharmacol. Sci. 14, 259-261 (1993)). Presynaptic GABA<sub>B</sub> autoreceptors have been shown to modulate GABA release, while heteroreceptors modulate the release of several neurotransmitters and peptides including L-glutamate, somatostatin, substance P, and cholecystokinin (Huston et al., Neurosci. 68, 465-478 (1995); Pende et al., Brain Res. 604, 325-330 (1993); Bonanno et al., Br. J. Pharmacol. 118, 1441-1446 (1996); Teoh et al., Br. J. Pharmacol. 118, 1153-1160 (1996); Raiteri et al., J. Pharmacol. Exp. Therapeut. 278, 747-751 (1996)). While the study of GABA<sub>A</sub> receptor structure and function has been advanced greatly by knowledge of their primary sequences, studies of the GABA<sub>B</sub> receptor have only recently begun. Kaupmann et al. (Nature 386, 239-246 (1997)) have described a sequence for a GABA<sub>B</sub> receptor which was isolated by expression cloning techniques. The identified receptors, rgb1a and rgb1b, are members of the G protein-coupled seven transmembrane domain receptor family and are splice variants of the same receptor. These receptors exhibit the expected sensitivity and rank order of potency of GABA<sub>B</sub> receptor compounds that has been described in the literature for GABA<sub>B</sub> receptors in tissue preparations.

20

### Summary of the Invention

The present invention relates to a new subtype of mammalian GABA<sub>B</sub> receptor which has been designated gb2 GABA<sub>B</sub>. In particular, the present invention relates to nucleic acids (DNA) encoding gb2 GABA<sub>B</sub>, to vectors and host expression systems containing these nucleic acids, to isolated and purified gb2 GABA<sub>B</sub> receptors, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and to antibodies produced against these antigens. Using expressed sequence tag (EST) data base searches, polymerase chain reaction, and library screening, we have identified and isolated human and rat cDNAs for a novel GABA<sub>B</sub> receptors, designated hgb2 and rgb2, with homology to rgb1a and rgb1b (the two alternatively spliced forms of gb1).

30

This is only the second GABA<sub>B</sub> receptor primary sequence to be identified. The homology between rat receptor sequences (rgb1 and rgb2) and human receptor sequence (hgb2) indicates that they might be members of a larger GABA<sub>B</sub> receptor family. These receptors (i.e., hgb2, rgb2, and rgb1) comprise a new subfamily of  
5 seven transmembrane G-protein-coupled receptors that share structure and sequence similarities with the metabotropic glutamate receptors. Baclofen and GABA modulate cAMP synthesis through the gb2 receptors at  $\mu$ M concentrations. The rgb2 mRNA exhibits a more discrete expression pattern in rat brain than rgb1 and appears to be absent from white matter suggesting that it is predominantly localized to  
10 neurons.

One object of the invention is to provide a nucleic acid sequence that encodes a protein which comprises a mammalian gb2 GABA<sub>B</sub> receptor. A further object of the invention is to provide a nucleic acid sequence that encodes a protein which comprises a human gb2 GABA<sub>B</sub> receptor.

15 A further object of the invention is to provide constructs containing the genes encoding such receptors under the transcriptional and translational regulatory control of regulatory genes recognized by a desired host to which the mammalian gb2 GABA<sub>B</sub> receptor genes are foreign.

A still further object of the invention is to provide an isolated or pure clone  
20 from a mammalian tissue DNA library, wherein the clone contains a DNA sequence that encodes a mammalian gb2 GABA<sub>B</sub> receptor. Another object of the invention is to provide an isolated or pure clone from a human tissue DNA library, wherein the clone contains a DNA sequence that encodes a human gb2 GABA<sub>B</sub> receptor.

Another object of the invention is to provide an expression vector that contains  
25 the DNA of the invention and is capable of transforming a host cell.

Still another object of the invention is to provide host cells and have been transformed with such DNA and which express a mammalian gb2 GABA<sub>B</sub> receptor.

Another object of the invention is to provide isolated or purified proteins comprising mammalian gb2 GABA<sub>B</sub> receptors. Still another object of the invention is  
30 to provide an isolated or purified protein comprising human gb2 GABA<sub>B</sub> receptor.

Yet another object of the invention is to provide methods for obtaining a DNA sequence encoding a mammalian gb2 GABA<sub>B</sub> receptor, methods for preparing a cell

that expresses the receptor, and methods for using such cells in the pharmacological, physiological, functional, or other investigational analysis of GABA<sub>A</sub> receptors, and agonists or antagonists for GABA<sub>A</sub> receptors.

To achieve the objects and in accordance with the purpose of the invention, as embodied and broadly described herein, isolated or essentially pure DNA sequences encoding mammalian gb2 GABA<sub>B</sub> receptors are disclosed herein. Preferably, the DNA sequences are cDNA sequences, and most preferably they are the cDNA sequences shown in SEQ. ID NO.: 1 (human) and SEQ. ID NO.: 2 (rat) or allelic variants thereof. Preferably the mammalian gb2 GABA<sub>B</sub> receptors are hgb2 GABA<sub>B</sub> receptors as defined by SEQ. ID NO.: 3 (human) and rgb2 GABA<sub>B</sub> receptors as defined by SEQ. ID NO.: 4 (rat) or allelic variants thereof.

Other objects and advantages of the present invention will be apparent from a consideration of the specification.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides schematic diagrams of hgb2 (panel A), rgb2 (panel B), rgb1b (panel C), and rgb1a (panel D) sequences and shows the relative location of oligonucleotide primers and restriction enzyme sites used in constructing the cDNAs and the location of oligonucleotide primers used to make riboprobes for *in situ* hybridization histochemistry. Left facing arrows represent sense primers, right facing arrows represent antisense primers. The cloning of hgb2 is detailed in Example 1; the cloning of rgb2 is detailed in Example 2; the cloning of rgb1b and rgb1a is detailed in Example 3.

Figure 2 illustrates the alignment of human gb2 and rat gb2 with rat gb1a and gb1b receptor amino acid sequences. Dots indicate residues identical in all receptors. Hydrophobic domains are denoted by bars and labeled with the transmembrane domain (TM) numbers. Potential N-linked glycosylation sites are designated by an asterisk over the appropriate residues.

Figure 3 illustrates hgb2 (panel A) and rgb1a (panel B) receptor-mediated modulation of forskolin-stimulated cAMP synthesis in hgb2/293-10 and rgb1a/293-18 cells. hgb2/293-10 and rgb1a/293-18 cells stably expressing the hgb2 and rgb1a receptors, respectively, were generated and assayed for modulation of forskolin-

stimulated cAMP synthesis as described in Example 8. Data are presented as the percent of total cAMP synthesized in the presence of forskolin only. Error bars represent the standard error of the mean for quadruplicate samples. The data presented are from a single representative experiment that has been replicated twice.

5 Figure 4 illustrates hgb2 mRNA expression in CNS and peripheral tissues as determined by Northern blot analysis. Clontech multiple human tissue Northern were hybridized with a probe directed against the hgb2 receptor. Weak bands were observed in heart and spinal cord human mRNA.

Figure 5 illustrates the localization of rgb2 and rgb1 receptor mRNA by *in situ* hybridization histochemistry. Coronal 12  $\mu$ m thin sections of a rat forebrain are shown. Brightfield (A and C) and darkfield (B and D) illumination is demonstrated of the identical visual fields; A and B show rgb1 and C and D show the rgb2 receptor mRNA. Note the lack of grains over the caudate nucleus and the septum in C and D. Abbreviations: c = caudate nucleus; cx = cerebral cortex; PO = preoptic area; s =  
15 septum. The scale bar represents 1 mm.

Figure 6 illustrates the localization of rgb2 and rgb1 receptor mRNA by *in situ* hybridization histochemistry. Coronal 12  $\mu$ m thin sections of a rat forebrain are shown. Brightfield (A and C) and darkfield (B and D) illumination is demonstrated of the identical visual fields; A and B show the rgb1 and C and D show the rgb2 receptor  
20 mRNA. Note the lack of grains over the hypothalamus in C and D. Abbreviations: Hi = hippocampus; Hth = hypothalamus; Th = thalamus. The scale bar represents 1 mm.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

25 The present invention relates to a new subtype of mammalian GABA<sub>B</sub> receptor which has been designated gb2 GABA<sub>B</sub>. In particular, the present invention relates to nucleic acids (DNA) encoding gb2 GABA<sub>B</sub> receptors, to vectors and host expression systems containing these nucleic acids, to isolated and purified gb2 GABA<sub>B</sub> receptors, to isolated and recombinantly-produced antigens encoded by these nucleic acids, and  
30 to antibodies produced against these antigens. Using expressed sequence tag (EST) data base searches, polymerase chain reaction, and library screening, we have

identified and isolated human and rat cDNAs for novel mammalian GABA<sub>B</sub> receptors, designated hgb2 and rgb2, with homology to gb 1a and gb 1b.

The DNA of the invention is an isolated or essentially pure DNA sequence (i.e., polydeoxyribonucleotide) encoding a protein which comprises the new  
5 mammalian subtype gb2 GABA<sub>B</sub> receptors. As used herein, the term "isolated" and variations thereof means that the DNA is in isolation from DNA encoding proteins normally accompanying these receptors or encoding a different GABA receptor. Thus, the DNA of the invention includes DNA encoding the gb2 GABA<sub>B</sub> receptor when that DNA has been cloned into a bacterial vector, such as a plasmid, or into a viral vector  
10 that may be harbored by a bacteriophage, provided that such clones are isolated from clones that contain DNA encoding other proteins normally accompanying such receptors or encoding a different GABA receptor. As used herein, the term "essentially pure" and variants thereof means the DNA is substantially free of DNA and RNA that does not encode the gb2 GABA<sub>B</sub> receptor. That is, there will be no  
15 more than about 0.1 percent of other DNA and RNA and preferably no more than about 0.01 percent of other DNA and RNA in any sample that contains the DNA encoding the gb2 GABA<sub>B</sub> receptor.

Preferably, the DNA of the invention is complementary DNA (cDNA). One preferred human cDNA has the nucleotide sequence as defined in SEQ. ID No.:1;  
20 another preferred rat cDNA has the nucleotide sequence as defined in SEQ. ID No.:2. The cDNA is approximately 5.5 to 5.8 kilobases in length. The preferred human and rat cDNA code for proteins with the deduced amino acid sequence of SEQ. ID NO.:3 and SEQ. ID NO.:4, respectively. These proteins contain seven transmembrane regions (TM1 through TM7 as shown in Figure 2B). As those skilled in the art will  
25 realize, allelic variations of the DNA and protein which do not significantly affect the functionality of the DNA and/or protein can also be employed and considered within the invention described herein. For purposes of this invention, an "allelic variation" of a given DNA and/or protein retains at least about 95 percent of the sequence of the comparison molecule (DNA or protein) and maintains the essential  
30 functionality of the comparison molecule. For the DNA of this invention, the essential functionality is encoding a mammalian GABA<sub>B</sub> receptor of subtype gb2; for the protein of this invention, the essential functionality relates to its action as a



GABA<sub>B</sub> receptor. Thus, such allelic variations may involve single or multiple mutations so long as the functionality remains intact.

The DNA of the invention may be obtained by various methods, as detailed in the examples, involving known molecular biology techniques. The present invention  
5 also encompasses isolated or purified clones from mammalian tissue DNA library, which clone contains a DNA molecule encoding a mammalian gb2 GABA<sub>B</sub> receptor. Preferably, the clones comprise an essentially pure culture of bacteriophage containing the cDNA of SEQ. ID. NO.: 1 or SEQ. ID. NO.:2 inserted into the genome  
10 of the phage. Alternatively, the clone comprises an essentially pure culture of bacteria, such as *E. coli*, containing the cDNA of SEQ. ID. NO.: 1 or SEQ. ID. NO.:2 inserted into a plasmid (e.g., pUC-18, pBSSK II (-), pcDNA/amp, pcDNA3.1, and the like) in the bacteria. Cosmids, BACE YAC vectors, and  $\lambda$  phages could also be used as expression systems for these genes.

The DNA of the invention can be used to transform procaryotic cells, such as  
15 bacteria, fungi, or other microorganisms, or transfect eucaryotic cells, such as yeast or, mammalian cells. Transformation or transfection is accomplished by techniques known in the molecular biology. The DNA of the invention maybe joined to a wide variety of other DNA sequences for introduction into an appropriate host cell. The companion DNA will depend upon the nature of the host, the manner of introduction  
20 of the DNA into the host, and whether episomal maintenance or integration is desired. Mammalian cells are the preferred host.

Generally, DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control  
25 nucleotide sequences recognized by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed or transfected by the vector. Therefore, it will be necessary to select for transformed or transfected host cells. One selection technique involves incorporating into the  
30 expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alter-

natively, the gene for such selectable trait can be on another vector, which is used to co-transform or co-transfect the desired host cell.

The preferred expression vector for use in the invention is the plasmid pcD3.1hgb2. This plasmid can be prepared from the expression vector pcDNA3.1.

5 After the modified expression vector has been prepared, it is transfected into a mammalian cell, using techniques known in the art. The mammalian cell may be any one that can be transfected with the DNA of the present invention.

Preferably, the cell is one that can be stably transfected with the DNA so that the DNA is integrated into the genome and expressed on an essentially continuous  
10 basis in the cell or its progeny. Further, the cell to be transfected is preferably one from a mammalian cell line that is capable of continuous growth in a suitable culture medium. Preferred types of cell lines include those derived from human embryonic kidney cells, such as HEK-293, neuronal cells, such as the type of pheochromocytoma cells known as PC 12 cells, fibroblast cells, such as A9 L cells, epithelial-like cells,  
15 such as Chinese hamster ovary (CHO) cells, and a glial or glial-like cells, such as c6 glioma cells.

After transfection, the cells are evaluated by screening to identify and isolate those that have taken up the expression vector. Preferably, the expression vector will be stably inserted into the genome of the cell. If the stably transfected cells were  
20 originally from a continuous cell line, the cell can be clonally expanded to provide a cell line that is capable of continuous growth in a suitable culture medium and which expresses a mammalian gb2 GABA<sub>B</sub> receptor.

A person skilled in the art, using known techniques and the teachings disclosed herein, can use the naturally variant or derived DNA of the invention to  
25 prepare sequencing vectors, expression vectors, transformed or transfected cells, and transfected cell lines as disclosed herein. Accordingly, these are within the scope of the present invention.

The cells and cell lines of the present invention can be used in the development of better drugs for the possible treatment of neurological and psychiatric  
30 disorders wherein GABA<sub>B</sub> receptors play an important role. The DNA, clones, and vectors of the present invention can be used for the construction of such cells and cell lines. Cells and cell lines that express mammalian gb2 GABA<sub>B</sub> receptors, particularly

those that express human gb2 GABA<sub>B</sub> receptors, will be extremely useful in the study of receptor function and its implications in neurological and psychiatric disorders. Relative to animal models, such cell culture assays are quick, inexpensive, and highly standardized. In addition, the cloned DNA sequences can be used to measure the  
5 mRNAs that encode these receptors, thus measuring tissue specific expression. Moreover, receptor pharmacology can be determined in isolation from other related receptors, including different GABA receptor types or subtypes. Therefore, these assays will be tools in the effort to provide a scientific basis for the rational development to new drugs.

10 In addition, the assays can be used to screen drugs in order to find or develop gb2 GABA<sub>B</sub> antagonists (substances that block the receptors) or agonists (substances that stimulate the receptors). Generally known techniques, modified in accordance with the discoveries and teachings disclosed herein, can be used with the cells and cell lines of the invention to evaluate potential antagonists or agonists by generating dose  
15 response, saturation, inhibition, or displacement curves. Initial candidates can be further tested for specificity, binding affinity, and activity.

Whether or not a chemical binds to a mammalian gb2 GABA<sub>B</sub> receptor can be determined by contacting the chemical with a cell or a part thereof from a host cell transformed by the DNA of the invention and determining the ability of the cell or  
20 part thereof to bind to the chemical in the appropriate assay in the presence of a chemical known to bind to a gb2 GABA<sub>B</sub> receptor, such as GABA and baclofen.

Gb2 GABA<sub>B</sub> receptor agonists are normally identified using both binding and functional assays. Such functional assays include the so-called second messenger assays. When neurotransmitters bind to receptors on the surface of cells, the  
25 metabolism of the various molecules is altered. When these molecules effect the physiology of the cell, they are referred to as second messengers. Two second messenger pathways that have been well characterized are inositol phosphate and cyclic adenosine monophosphate (cAMP) metabolism. See Berridge, Annu. Rev. Biochem. 56, 159 (1987) and Gilman, Annu. Rev. Biochem. 56, 615 (1987). cAMP  
30 metabolism can be examined by various methods. The activity of the enzyme adenylate cyclase or the levels of cAMP can be measured. Thus, the invention provides an in vitro method for evaluating a chemical to determine if it is a gb2

GABA<sub>B</sub> receptor agonist. The chemical is contacted with a cell transformed by the DNA of the invention. The effect of the chemical on a second messenger pathway of the cells is then measured using conventional techniques.

Cells and cell lines expressing mammalian gb2 GABA<sub>B</sub> receptors can be used to provide a sufficient amount of the proteins for the preparation of antibodies using conventional techniques. The antibodies can be used to define more clearly the localization of receptor types than is possible using in situ hybridization to mRNA. They could also be used for diagnostic purposes if clinically significant abnormalities were found in association with the gb2 GABA<sub>B</sub> receptors. Moreover, they would permit purification of the receptor proteins by affinity chromatography. Highly purified gb2 GABA<sub>B</sub> would permit the 3-dimensional protein to be studied by X-ray crystallography. Such information would be especially useful in rational drug design.

The molecular biology techniques used in making and using the present invention are generally known in the art. For example, suitable techniques and procedures are generally described in "Molecular Cloning: A Laboratory Manual," 2<sup>nd</sup> Ed., Sambrook, Fritsch, and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, N.Y., 1987 (updated quarterly).

**Hgb2 and rgb2 Amino Acid Sequences.** Through EST data base searches, polymerase chain reaction, and library screening as described in the Examples, human and rat homologues of a novel receptor (hgb2 in human and rgb2 in rat) have been identified and isolated. These receptors have homology to the GABA<sub>B</sub> receptors (rgb1a and rgb1b from rat) identified by Kaupmann et al. (Nature 386, 239-246 (1997)). Figure 1 shows a schematic of the hgb2, rgb2, rgb1a and rgb1b receptor nucleotide sequences denoting the placement of oligonucleotide primers and restriction enzyme sites used to generate each of these constructs and the expression plasmids. Hgb2 receptor fragments were amplified from human brain cDNA while rgb2 was isolated from a rat cortex cDNA library followed by amplification of rat brain cDNA to obtain the 5' end of the receptor including the start site. The resulting 5786 base pair sequence generated for hgb2 includes an open reading frame of 2826 bases. The combined 5' RACE (i.e., rapid amplification of cDNA ends) product and insert for rgb2 spans 5459 base pairs and includes an open reading frame of 2823

bases. The cDNA sequences for hgb2 and rgb2 predict proteins of 942 and 941 amino acid, respectively, with relative molecular mass of 104 kD.

Sequence similarity and hydropathy profiles (Kyte et al., J. Mol. Biol. 157, 105-132 (1982) of the predicted proteins demonstrate that they share structural features with the GABA<sub>B</sub> receptor rgb1 (Kaupmann et al., Nature 386, 239-246 (1997)), the parathyroid cell calcium-sensing receptor, and the family of metabotropic glutamate receptors (for review see Pin and Duvoisin, Neuropharmacol. 34, 1-26 (1995)). Significant features in the hgb2 and rgb2 sequences include 7 putative transmembrane regions, a large extracellular amino terminus of 476 residues, and 4 potential sites for N-linked glycosylation (Figure 2). There is an abundance of proline residues in the amino terminus of both hgb2 and rgb2, with a string of 9 proline residues only 9 residues into the sequences.

Comparison of the amino acid sequences for hgb2 and rgb2 with that for rgb1a and rgb1b (Figure 2) shows 28% amino acid identity which rises to 38% when conservative amino acid substitutions are taken into account. Homology between the gb2 and gb1 receptors is not limited to the hydrophobic regions but instead spans the entire protein. Search of Genbank indicated that hgb2 and rgb2 share significant sequence homology with the metabotropic glutamate receptors. The Genbank search also showed a limited degree of homology between the amino acid sequence of hgb2 and rgb2 and the human and rat calcium-sensing receptors.

**Hgb2 Encodes a GABA<sub>B</sub> Receptor.** A functional screen of HEK293 cells stably transfected with pcD3.1hgb2 or pcD3.1rgb1a revealed several cell lines expressing these receptors to varying degrees. Preliminary data is shown in Figure 3 for the stable cell lines hgb2/293-10 and rgb1a/293-18. Adenylyl cyclase was stimulated as described in the Example 8 with 10 uM forskolin in the presence or absence of 30 uM baclofen or 30 uM GABA, 100 uM AOAA (a GABA transaminase inhibitor), and 100 uM nipecotic acid (a GABA uptake blocker). hgb2/293-10 cells exhibited a 65% reduction in forskolin-stimulated cAMP synthesis with baclofen and a 50% decrease with GABA plus AOAA and nipecotic acid (Figure 3). A 40% reduction in forskolin-stimulated cAMP synthesis was observed for rgb1a/293-18 cells treated with baclofen and a 45% decrease was observed for GABA plus AOAA and nipecotic acid (Figure 3). Neither baclofen nor GABA plus AOAA and nipecotic

acid in the absence of forskolin had any affect on cAMP synthesis (Figure 3). These data suggest that the hgb2 receptor, like the rgb1a and rgb1b receptors, is a GABA<sub>B</sub> receptor since the criteria for baclofen sensitivity has been established. Kaupmann et al. (Nature 386, 239-246 (1997)) observed similar reduction in forskolin-stimulated cAMP synthesis with HEK293 cells stably expressing the rgb1a receptor. However, it is worth noting that it took 300 uM baclofen to observe a 30% decrease in forskolin-stimulated cAMP synthesis as compared to the 30 uM used in the current study which accomplished a 40% reduction. These differences may be due to differences in the levels of receptor expression between the rgb1a stable cells and rgb1a/293-18 cells.

Acquisition of a radiolabeled antagonist for binding analyses will be necessary before differences in receptor cell number may be addressed. Work is underway to further characterize the pharmacology of these receptors and to establish a rank order of potency for GABA<sub>B</sub> receptor agonists and antagonists.

**Rgb2 Expression in Rat CNS and Peripheral Tissues.** Analysis of Northern blots of human mRNA from several brain regions and peripheral tissues with a probe directed against the hgb2 receptor reveals a single mRNA species of 6.2 kb (Figure 4). Hgb2 receptor mRNA is expressed in all of the brain areas tested although the level of expression seems to vary significantly with the highest being found in the cortex and thalamus and the lowest in the corpus callosum, caudate nucleus, and medulla. In addition, a weak 6.2 kb band is detected in spinal cord mRNA. No hgb2 receptor mRNA is detected in any of the peripheral tissues examined with the exception of the heart where a weak band is observed.

In situ hybridization histochemistry was carried out using sense and antisense probes directed against both the rgb2 and rgb1 receptors for comparison as described in the Examples. The rgb1 receptor probe is directed against a region common to both the rgb1a and rgb1b receptors (see Examples and Figure 1) therefore mRNA for both receptors will be hybridized. Rgb1 receptor mRNA is heterogeneously expressed in abundance throughout all regions of the rat brain as previously described (Kaupmann et al., Nature 386, 239-246 (1997)). Rgb2 receptor mRNA is also abundant in rat brain, but its distribution is somewhat more restricted. There are significantly lower levels of rgb2 receptor mRNA hybridization in the caudate-putamen, septum, medial

basal hypothalamus, and brainstem of the rat brain as compared with levels of rgb1 receptor mRNA (Figures 5 and 6). The levels and distribution of rgb2 and rgb1 receptor mRNA hybridization in all other brain regions examined appeared to be nearly identical. In contrast to rgb1 receptor mRNA, hybridization to rgb2 receptor mRNA was not detected in white matter, suggesting that expression of rgb2 receptor mRNA is restricted to neurons. Kaupmann et al. (Nature 386, 239-246 (1997)) showed that rgb1 mRNA is expressed in neurons but not glia in the CA3 field of the hippocampus. Detailed examination of other brain regions has revealed that rgb1 mRNA is found in glia as well. While hybridization to rgb2 mRNA has not been detected in glia, more detailed studies of rgb2 mRNA expression and protein localization at the cellular level are necessary before this possibility can be ruled out.

While it is difficult to make a direct extrapolation from mRNA hybridization to protein location, the data presented herein are generally in agreement with previously published binding data (Chu et al., Neuroscience 34, 341-357 (1990); Bowery et al., Neuroscience 20, 365-383 (1987)). Binding of GABA in certain regions of the rat brain including the septal area, caudate putamen, hypothalamus and brainstem, with the exception of the interpeduncular nucleus, is relatively low (Chu et al., Neuroscience 34, 341-357 (1990); Bowery et al., Neuroscience 20, 365-383 (1987)) in agreement with the levels of hybridization which were detected for rgb2.

The following examples are intended to illustrate the invention and not to limit the invention. All references cited in the present specification are hereby incorporated by reference.

**Example 1 -- Cloning of hgb2.** A tblastn search of the Genbank EST database using the amino acid sequence of the rat gb1 receptor as query detected three ESTs (R76139, R80448, and Z43654) which showed distant homology to transmembrane domains 5-7 and another EST (T07261) which showed similarity to transmembrane domains 1-3. Primers designed from these sequences (JC136 gcgggatccCTTCGGCACGAATACCAGGCAGAGGGT for 5' extension; JC137 ccggaattcCAGAAGCTCATAAAGATGTCTGAGTCC and JC135 ccggaattcTTCTTAGCTTTGGGAGACCCGCAACGT for 3' extension) were used

- with the Clontech API primer to do 5' and 3' RACE using Clontech Marathon-Ready human brain cDNA as template and touchdown PCR conditions (94°C, 30 sec and 72°C, 4 min for 5 cycles; 94°C, 30 sec and 70°C, 4 min for 5 cycles; 94°C, 30 sec and 68°C, 4 min for 20 cycles). JC135 and JC136 primers were derived from the transmembrane domains 5-7 ESTs while JC137 was derived from T07621. JC136 and JC137 primers were used together to generate a product of 722 bases which, when sequenced, confirmed that the ESTs were derived from the same mRNA. 3' products from RACE with JC135 and JC137 were reamplified with the Clontech AP2 primer yielding two products of 1.2 and 1.7 kb in size but lacking a poly(A) signal.
- 10 A tblastn search of the Genbank EST database with the 3' sequences detected an EST (AA323988) which overlapped with the query sequence and extended the sequence through an A-rich region. Gb2-15 (AGCACTAGAACTCCAGCTGGAAGTCA) was designed based on EST AA323988 and used in a 3' RACE reaction as described above to yield a 2.3 kb band that when sequenced was shown to include a poly(A) signal. 5' RACE with JC136 yielded a 0.88 kb Not I/BamH I fragment which when sequenced showed weak amino acid homology to rgb1. JC153 (GCCTACGATGGCATCTGGGTCATCGC) was designed based on the JC136 5' RACE product sequence and paired with JC136 to yield a 1.1 kb fragment. Further 5' extensions were obtained by using Clontech Advantage-GC PCR (1 µl GC-melt per 50 µl reaction, PCR conditions: 94°C, 15 sec and 68°C, 3.5 min for 30 cycles) and nested primers JC152 (CAGATTCCAGCCTTGGAGGGACTCTGC) followed by JC186 (TGCATCGTAGAAGGCTTTCAACCCTTTTGC) and adaptor primers API followed by AP2.
- 25 pcDhgb2I was generated by subcloning a Nae I/Xho I fragment from a JC153/JC165 (JC165: AATAAGGCTCGAGGTCAGGTGCCAA) 2.2 kb PCR product and an EcoR I/Nae I fragment from a JC171/JC154 (JC171: CAGCAGCCCGCCGCTCTCCATCAT; JC154: gcgcgtaatacgaactcactatagggGTAGTTGAAGTCCTGGATCC G) 1 kb PCR product into EcoR I/XhoI digested pcDNA1/amp (Invitrogen). The expression construct pcDhgb2II was constructed by subcloning a Not I/Xmn I 5' RACE fragment obtained with AP2/JC186 into a Not I/Xmn I digested pcDhgb2I. PcD3.1hgb2 was made by
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subcloning an EcoR I/Xba I fragment from pcDhgb2 into EcoR I/Xba I digested pcDNA3.1 (Invitrogen).

**Example 2 -- Cloning of rgb2.** Rgb2 was obtained by screening pools of clones from a rat cortex cDNA library using Southern blot analysis. DNA was prepared from each of 49 pools representing an average of  $5 \times 10^5$  clones per pool and digested with Xho I to release the inserts from the vector DNA. 5  $\mu$ g of Xho I digested pool DNA was run on a 0.8 % agarose gel and transferred to a nitrocellulose supported membrane (BA-S 85, Schleicher & Schuell) using a TurboBlotter (Schleicher & Schuell). Nitrocellulose membranes were hybridized with a  $^{32}$ P-labeled random primed (Boehringer Mannheim) hgb2 JC137/JC136 (JC137: ccggaattcCAGAAGCTCATAAAGATGTCGAGTCC; JC136: gcgggatccCTTCGGCACGAATACCAGGCAGAGGGT) PCR fragment in 3x Denhardt's and 3x SSC at 60°C. Hybridization was followed by two 15 min washes in 3x SSC at 60°C. Blots were imaged on the Fujix PhosphorImaging system (Fuji). Bacteria from two pools was each used to make 22 subpools. DNA was prepared from these subpools and analyzed as described above. Four positive subpools (10I, 10M, 37O, and 37Y) were analyzed further in subpools representing  $3 \times 10^3$  clones per subpool. Nitrocellulose filter lifts were made of successive platings of subpools 10I7 and 37O9 and processed following standard protocols ("Molecular Cloning: A Laboratory Manual," 2<sup>nd</sup> Ed., Sambrook, Fritsch, and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, N.Y., 1987 (updated quarterly)). Filters were hybridized as described above followed by two washes with 3x SSC at 60°C for 15 min and 1 wash in 3X SSC at 60°C for 1 hr. Filters were exposed to BioMax MR film (Kodak) for 16 hr.

Two positive overlapping clones were isolated from this screen, 10I75a and 37O94a. The 5200 kb clone 37O94a was sequenced and found to lack a start site. RACE was performed using the high GC conditions described above with nested primers JC190 (CGCTGGTGCAGGGATCATTGGAGAA) and JC191 (CATATTCTGGTCAAAGTGGCCAAGG) paired with AP1 followed by AP2 using Clontech Marathon-Ready rat brain cDNA as template to yield the 5' end of the rgb2

receptor. A Xho I/Bgl II fragment from the 5' RACE of *rgb2* and a Bgl II fragment from 37094a were subcloned into Xho I/BamH I digested pBSSK II (-). The correct orientation of the Bgl II fragment was established using restriction enzyme mapping with XhoI and BamH I. PcD3.1*rgb2* was made by subcloning a Kpn I/BamH I fragment of the pBSSK II (-) plasmid with a BamH I/Xba I fragment from 37094a into Kpn I/Xba I digested pcDNA3.1.

**Example 3 -- Cloning of *rgb1b* and *rgb1a*.** *Rgb1b* was obtained by amplification of Clontech rat brain Marathon-Ready cDNA with JC140 (ccggaattccaccATGGGCCCCGGGGGACCCTGTACC) and JC141 (CTAGTCTAGATCACTTGTAAGCAAATGTACTCGACT). pBS*rgb1b* was made by ligation of an EcoR I/Xba I fragment of *rgb1b* into EcoR I/Xba I prepared pBSSKII(-). pcD*rgb1b* was constructed by subcloning an EcoR I/Not I fragment from pBS*rgb1b* into EcoR I/Not I prepared pcDNA1/amp (Invitrogen). *Rgb1a* 5' sequence was obtained by 5' RACE with AP1 or 2 paired with JC144 (GAGTAG TTCGTACAAGTACTTGGTGGC) using Clontech rat brain Marathon-Ready cDNA as template. An *rgb1a* 5' fragment for vector construction was generated by amplification of the JC144 RACE product with JC139 (ccggaattccaccATGCTGCTGCTGCTGCTGGTGCCTCTC) and JC144. pBS*rgb1a* was made by ligation of an EcoR I/Hpa I fragment from the JC139/JC144 PCR product into EcoR I/Hpa I digested pBS*rgb1b*. pcD*rgb1a* was constructed by subcloning an EcoR I/BstE II fragment from the pBS*rgb1a* into EcoR I/BstE II digested pcD*rgb1b*. An EcoR I/Not I fragment from pcD*rgb1a* was subcloned into pcDNA3.1 (Invitrogen) to make pcD3.1*rgb1a*.

**Example 4 -- Northern Analysis.** Clontech multiple human tissue Northern were hybridized with the 0.88 kb Not I/BamH I fragment (bases 732-1613 of *hgb2*) at 42°C in 5X SSPE, 10X Denhardt's, 50% formamide, 2% SDS, 100 µg/ml denatured salmon sperm DNA. Following hybridization blots were washed three times for 10 min each in 0.2X SSPE, 1% SDS at 60°C. Blots were imaged on the Fujix PhosphorImaging system (Fuji).

**Example 5 -- In Situ Hybridization Histochemistry.**

Preparation of rat brain sections. prehybridization and hybridization of rat brain slices was performed using conventional techniques (see, for example, Bradley et al., J. Neurosci. 12, 2288-2302 (1992); a general protocol can also be found on the following website:

- 5 <http://intramural.nimh.nih.gov/lcmr/snge/Protocol.html>). Sections were hybridized with labeled antisense and sense riboprobes directed against rgb2 or rgb1. Probes were generated by amplification of rgb2 with JC216 paired with JC217 or with JC218 paired with JC219 (JC216:
- cgcgcaattaaccctcactaaaggACAACAGCAAACGTTTCAGGC; JC217:
- 10 cgcgtaataacgactcactatagggCATGCCTATGATGGTGAG; JC218:
- cgcgcaattaaccctcactaaaggCTGAGGACAAACCCTGACGC; JC219:
- cgcgtaataacgactcactatagggGATGTCTTCTATGGGGTC) or by amplification of rgb1 with JC160 paired with JC161 (JC160:
- cgcgcaattaaccctcactaaaggAAGCTTATCCACCACGAC; JC161:
- 15 cgcgtaataacgactcactatagggAGCTGGATCCGAGAAGAA) and labeled as described previously (Bradley et al., J. Neurosci. 12, 2288-2302 (1992); the general protocol can also be found at <http://intramural.nimh.nih.gov/lcmr/snge/Protocol.html>).

**Example 6 -- Cell Culture and Stable Expression of hgb2 and rgb1a.**

- 20 HEK-293 cells, human embryonic kidney cells, were cultured in DMEM supplemented with 10% fetal calf serum (Sigma), 1% penicillin/streptomycin (GIBCO/BRL), at 37°C in 5% CO<sub>2</sub>. HEK-293 cells grown to 75% confluency in 10-cm tissue culture plates were used for control, pcD3.1hgb2 and pcD3.1rgb1a transfections. HEK-293 cells were exposed to either a solution of cationic lipid/DNA
- 25 (LipofectAMINE; GIBCO/BRL) in DMEM without fetal calf serum or penicillin/streptomycin (70 µg LipofectAMINE/10 µg of pcD3.1hgb2 or pcD3.1rgb1a) or a solution containing cationic lipid alone (70 µg LipofectAMINE) in DMEM, for 5 hr at 37°C. After incubation, lipid/DNA solutions were supplemented with equal volumes of DMEM with 20% fetal calf serum and cells were set at 37°C
- 30 overnight. Within 24 hr of transfection media was removed and replaced with serum- and penicillin/streptomycin-supplemented DMEM. Transfected cells were allowed to recover for 24 hr after which each 10-cm plate was split to five 10-cm plates in

DMEM supplemented with 10% fetal calf serum, 1% penicillin/streptomycin and 375  $\mu\text{g/ml}$  geneticin (G418; and GIBCO/BRL) for a period of 2-4 weeks. Resistant colonies were isolated and transferred to 24-well plates. Those cells that survived the isolation procedure were cultured for preparation of total RNA for dot blot analysis (Example 7) and for cAMP response assays (Example 8) to screen for expression of hgb2 and rgl1a.

**Example 7 -- Detection of hgb2 and rgl1a RNA by Dot Blot Analysis.** The cells from Example 6 were grown in 6-well plates to 75-100% confluency. Total RNA was prepared using the protocol provided with Trizol (GIBCO/BRL). Briefly, Trizol was added to each well and cells were triturated and transferred to 1.5 ml microfuge tubes. Samples were extracted with chloroform followed by precipitation with 2-propanol. Pellets were resuspended in 7.4% formaldehyde/6X SSC and stored at  $-20^{\circ}\text{C}$  until analysis. Half of each sample was diluted in equal volume of 7.4% formaldehyde/6X SSC, denatured and applied to a supported nitrocellulose membrane using a dot blot apparatus. RNA was fixed to the membrane by UV crosslinking (Stratalinker, Stratagene) and blots were prehybridized in 6X SSC, 5X Denhardt's, 0.5% SDS, 50% formamide and 100  $\mu\text{g/ml}$  denatured salmon sperm DNA at  $42^{\circ}\text{C}$  for at least 2 hr. Blots were hybridized with  $^{32}\text{P}$ -labeled random primed PCR fragments for hgb2 and rgl1a (hgb2: JC137/JC136; rgl1a: JC160/JC161),  $1-5 \times 10^6$  cpm/ml, were denatured and added directly to the prehybridized solution. Blots were hybridized at  $42^{\circ}\text{C}$  overnight followed by one 20 min wash at  $25^{\circ}\text{C}$  with 2X SSC, 0.1% SDS and two 30 min washes at  $68^{\circ}\text{C}$  with 0.2X SSC, 0.1% SDS. Blots were imaged on the Fujix PhosphorImaging system. Data tabulation is underway.

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**Example 8 -- Determination of Cellular cAMP Response.** HEK-293 cells from Example 6 which stably express the hgb2 and rgl1a receptors were lifted in 1X PBS, 2.5 mM EDTA, counted, pelleted and resuspended at  $1.5 \times 10^5$  cells per 100  $\mu\text{l}$  in Krebs-Ringer-Hepes medium, 100  $\mu\text{M}$  Ro 20-1724 (RBI; Natick, MA) and incubated at  $37^{\circ}\text{C}$  for 20 min. 100  $\mu\text{l}$  cells was added to 100  $\mu\text{l}$  of prewarmed ( $37^{\circ}\text{C}$ , 10 min) Krebs-Ringer-Hepes medium, 100  $\mu\text{M}$  Ro 20-1724 without or with agonists, antagonists and/or forskolin. Following a 20 min incubation at  $37^{\circ}\text{C}$  the assay was

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terminated by setting the cells on ice and centrifuging at 2000 rpm for 5 min at 4°C. 175 µl of assay solution was removed and replaced with 175 µl of 0.1 N hydrochloric acid. 0.1 mM calcium chloride and cells were set on ice for 30 min and then stored at -20°C. cAMP determinations were made using a solid phase modification (Maidment et al., *Neurosci.* 33, 549-557 (1989)) of the cAMP radioimmunoassay described by Brooker et al., *Adv. Cyclic Nucleotide Res.* 10, 1-33 (1979). Immulon II removable wells (Dynatech; Chantilly, VA) were coated overnight with 100 µl of protein G (1 mg/ml in 0.1M NaHCO<sub>3</sub>, pH 9.0) at 4°C. Prior to use protein G-coated plates were rinsed with PBS-gelatin-Tween (phosphate buffered saline containing 0.1% gelatin, 0.2% Tween-20) 3 times quickly and then once for 30 minutes. Following the rinse with PBS-gelatin-Tween the RIA was set up by adding 100 µl 50 mM sodium acetate, pH 4.75, cAMP standards or aliquots from treated cells, 5,000-7,000 cpm [<sup>125</sup>I]-succinyl cAMP, and 25 µl of a sheep antibody to cAMP diluted in 50 mM sodium acetate, pH 4.75 (Atto instruments; dilution of stock to 2.5x10<sup>-6</sup>, determined empirically) to the plates in a final volume of 175 µl. Plates were incubated 2 hr at 37°C or overnight at 4°C, rinsed 3 times with sodium acetate buffer, blotted dry, and then individual wells broken off and bound radioactivity determined in a gamma counter. EC<sub>50</sub> values were determined by nonlinear least squares fit of the data to the equation

$$Y = A_0 + (A_m * L) / (EC_{50} + L)$$

where Y is the amount of cAMP (picomole/well), A<sub>0</sub> is the basal level of cAMP, A<sub>m</sub> is the maximal accumulation under these conditions, L is the added ligand concentration, and EC<sub>50</sub> the apparent half-maximal concentration for stimulation of cAMP accumulation. Data tabulation is underway.

## SEQUENCE LISTING

## (I) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Mammalian gb2 GABA<sub>B</sub> Receptors

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(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: Windows

(D) SOFTWARE: FastSEQ for Windows Version 2.0

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5786 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTGTTCTTAT	CGGATGCTCC	CAGTAACCAG	GGGGACCACC	CGAGCTAATC	ATGGAATGTC	4140
TGTTCCCGAG	AAACACGATA	AAGAAAGATT	GTGCACTTTA	ACCTCTCTCA	TCAGGGCCCA	4200
AGGGCTGGCT	GGGATTTTTT	TTTTTTTTTT	TTCCCACTAA	CTTTGTTTCT	GACCAAAGTG	4260
AATTGGAGGC	ATTCTGCTAA	AAGACATCCC	CGTAGACATA	GGGGAGAGAG	TTGCTGGCTG	4320
AGGGCTTCCC	TTGGCTTCCA	GAAGGCAGCC	TTCCATCCAG	ACAAGCCAGT	GAGCTCTCCC	4380
CTTGGGATCA	CTGGGGTGAT	CAGTCAGCAG	ATTGATTCTC	ATTACATAAG	TCATTCTCTC	4440
CTTTAAATTG	AGCCCTAAG	AGCACTGGCC	TGGGAGTCAG	ACAGACCTGG	GTTCAAGTCC	4500
TCAGTCCCTT	GCCCACTCCC	TACGTGACTT	TGATCAGGTC	ACTAGTGTCT	CTCTGAGCCT	4560
CAGTTTCCCC	TCTGTAACTT	GGGGTTGAAC	TAAAAACCTT	GTCTGCCTTA	CCTCACAAAG	4620
TCACTCTGAG	GATTGAAACT	TGATCTTGTC	CAGGAAAGCT	TTGTACCAAA	CAGTGAAGCC	4680
GCCCTGATCC	GTGAGGTATG	AGTATGACTC	TGACCTTCAG	CCCTCCCTAC	AGCCGGGGGT	4740
GTGGCCCAAG	GAAGCTTCCA	GCACAGCCCT	CTACCCAGAA	CATCCGGGCT	GGAGGGAGGC	4800
TCCCAGTGAC	TTTTCTGACA	TTCTAGACA	GGTTCATTCT	TTGCTCAAGA	AAGGCCTGAA	4860
TGACAATGTC	CAGGATGTCT	GCACAACTGA	GCAGCTCGCT	CACTCCCTAA	AGAAACCTAT	4920
TGGCAGCTTC	AACAGGCAGG	CAATAATCTC	TTCCAGAAC	CACTGCAGTC	AGGAATAAAC	4980
TGTTTTCTCC	ACCAGGCTTT	GACAAAAGGG	CCACAGGAA	TCTTACCAAT	GCCAACATTT	5040
CAAAGCAGCC	TATTTACAGT	AGCATAGCTT	TCTGCTCCCC	TTCCCCAAAG	AGAGGTTATG	5100
GAGGTACTGT	AGCTTTTAGG	GAAAAAAGAA	ATGTTAACAC	ATCACAGGTC	AAGTTGAAGT	5160
CATTCTCTGT	TTAGGCACTA	AAAATCGGTG	TTGTCACCTA	CTGTGTATTA	CCAGTATTTA	5220



CTTGCTTTCT	TGATTTGACC	AAAACCAAAT	TTAATTTAAA	GGACCACATT	AATTTTTCAA	5280
AGGGAAAGAG	ACAATTAATT	GTACATAATG	TATACACACA	CAAAAAAAAA	AATACCTGTA	5340
GAAATATTAT	TCCAGCATAG	CAGGAAAACA	AACAAAAGTA	TTGGACTCTC	GGAGGTGAGC	5400
CTGTGCGTCT	GTAACCCCTT	GTGACTCCTG	AGCGTGCGCT	GTCTTCTAGG	TTAACTCACG	5460
AAGTACATTC	TCTGTCTTAC	TGATACTGTA	GGTTCACCCA	TTTTTTTTTA	ATTTCTCTGC	5520
AAATAACAAG	ACCCACAGAA	GTGACTCTAG	CTACTTAATG	GTTCTGTTCT	TTTATATGCA	5580
GCAAAACACAC	CGTCCATTTT	TGAAGAGGCT	TCGGCCTGAA	GGCATTITTC	AATGATGTTA	5640
GTGCACAAAA	CGCTTTAAAT	TAGACTGGAA	CTGCCAGAAT	CAAAATGTAA	TGAGGAATTT	5700
CTCGTACCCC	TACTGCATGG	TATCGATTTT	TAATAAATTG	TTGCAAATTT	GTTTTTATGA	5760
ATAAAAGGAA	AAAACCTGTC	GTCITT				5786

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5459 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GGCGTTCTG AGCGAGCAGA AGCCGGAGCC GAGCCGGGGC CCGTGCCGGC GCCATTGCGC   60
GGGRGCCGCG GGCAAAKCTC GGGGCCGGGC GGGGGGCCGG GCCAGGCCAT GCGGGCCGAG   120
TGAGCTGGCG CCCGAGCCCG GCGGCGCGGC ATGGCTTCCC CGCGAGCTC CGGGCAGCCC   180
CGCGCGCGCG GCGGCGCGGC GCGGCGCGGC CGGCTGCTGC TCGCGCTGCT GCTGCTGGTG   240
CTGCTGTGCT TGGCGCGCGG GCGCTGCGGC TGGACGCGCG GCGCGCGCGC GCGCGCGCGC   300
AGCAGCCCGC CGCTCTCCAT CATGGGCGCTC ATGCGGCTCA CCAAGGAGGT GGCCAAGGGC   360
AGCATCGGGC GCGGCGTGCT CCGCGCGCTG GAGCTAGCCA TCGAGCAGAT CCGCAACGAG   420
TCACTCCTGC GCGGCGTACT CCGGCGCTG CGACTCTATG ACACCGAGTG TGACAATGCA   480
AAGGGACTGA AAGCCTTCTA TGACGCAATA AAGTATGGGC CGAACCATTG GATGGTGTTT   540
GGAGGCGTCT GTCCGTCTGT CACATCTATT ATCGCGGAGT CCCTCCAAGG CTGGAATCTG   600
GTGCAGCTTT CCTTCGCGCG CACCACGCGT GTTCTTGGCG ATAAGAAGAA GTACCCGTAT   660
TTCTTCGCGA CGGTGCGCGC AGACAACGCG GTGAACCCCG CCATCCTGAA GCTCCTGAAG   720
CACTTCGCGT GCGGCGGTGT GGGCACATC ACGCAGGAGC YGCAGCGCTT CTCCGAGGTG   780
AGGAATGACC TGACTGGGGT TCTGTATGGG GAAGATATTG AGATCTCAGA CACAGAGAGT   840
TTCTCCAATG ATCCTTGAC CAGCGTCAAA AAGCTCAAGG GGAATGACGT GCGGATCATC   900
CTTGCGCAGT TTGACCAGAA TATGGCAGCA AAAGTCTTCT GTTGTGCTT CGAGGAGAGC   960
ATGTTTGCCA GCAAGTACCA GTGGATCATC CCGGGATGGT ACGAGCCTGC GTGGTGGGAG   1020
CAGGTGCATG TGGAGGCCAA TTCCTCAGCG TGCTGCGCA GAAGCCTCCT GGCTGCCATG   1080
GAAGGTTACA TCGGAGTGGA CTTTGAGCCC CTGAGCTCCA AACAAATCAA GACCATCTCA   1140
GGGAAGACTC CACAGCAGTT TGAAGAGAG TACAACAGCA AACGTTCAAG CGTGGGGCCC   1200
AGCAAGTTCC ATGGGTACGC CTACGATGGG ATCTGGGTCA TCGCCAAGAC CCTACAGAGG   1260
GCCATGGAGA CACTGCATGC CAGTAGCAGG CACCAGCGGA TCCAGGACTT CAACTACACA   1320
GACCACACGC TGGGCAAAAT CATCTCAAT GCCATGAACG AGACCAACTT CTTCGGGGTC   1380
ACGGGTCAAG TTGTGTTCCG GAACGGGGAG AGAATGGGAA CCATTAAATT TACTCAATTT   1440
CAAGACAGCA GAGAGGTGAA GGTGCGCGAA TACAACGCGG TGGCTGACAC ACTGGAGATC   1500
ATCAATGACA CCATAAGGTT CCAGGGGTCC GAGCCACCCA AGGACAAGAC CATCATTCTG   1560
GAGCAGCTTC GGAAGATCTC GCTTCCACTG TATAGCATCC TGTCGCTCT CACCATCCTC   1620
GGCATGATCA TGGCCAGCGC CTTCCTCTTC TTCAACATCA AGAACCGGAA CCAAAAGCTG   1680
ATTAAGATGT CAAGCCCCTA CATGAACAAC CTCATCATCC TGGGAGGAAT GCTGTCTTAT   1740
GCATCCATCT TCCTCTTTGG CCTCGACGGG TCCTTCGTCT CAGAAAAGAC CTTTGAACA   1800
CTCTGCACGG TCGGACCTG GATTCTCACC GTGGGCTACA CAACTGCCTT TGGGGCCATG   1860
TTTGCAAGA CTTGGAGGGT CCATGCCATC TTCAAAAATG TGAAGATGAA GAAGAAGATC   1920
ATCAAGAGCC AGAAGCTGCT TGTGATTGTG GGGGGCATGC TGCTCATCGA CCTGTGCATC   1980
CTGATCTGTT GGCAGGCTGT GGACCCCTG CCGAGGACAG TGGAGAGGTA CAGCATGGAG   2040
CCGGACCCAG CAGGCGGGA CATCTCCATC CGCCCATTCG TGGAACTG CGAAAACACC   2100
CACATGACCA TCTGGCTTGG CATTGTCTAC GCCTACAAGG GGCTCCTCAT GCTATTCGGT   2160
TGTTTCTTGG CATGGGAAC CCGCAATGTG AGCATCCCTG CCCTCAACGA CAGCAAGTAC   2220
ATCGGCATGA GTGTGTACAA TGTGGGATC ATGTGCATCA TCGGGGCTGC TGTCTCCTTC   2280
CTGACGCGTG ACCAGCCCAA CGTGCACTTC TGATCGTGG CCCTGGTCAT CATCTTCTGC   2340
AGCACCATCA CTCTCTGCTT GGTGTTTGTG CCAAAGCTCA TCACCTGAG GACAAACCCT   2400
GACGCAGCCA CTCAGAACAG GCGTTCAG TTCACACAGA ACCAGAAGAA AGAAGATTGC   2460

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AAGACCTCCA GTTCAGTCAC CAGCGTGAAC CAGGCGAGCA CGTCACGGCT GGAGGGACTG 1530  
 CAGTCAGAAA ACCACCGCCT TCGAATGAAG ATCAGAGAGC TGGACAAAGA GTTGAAGAA 1580  
 GTCACCATGC AGCTACAAGA CACACCAGAG AAGACCACAT ACATCAAACA GAATCACTAC 1640  
 CAAGAGCTCA ACGACATCCT CAGCTTGGGC AACTTCACAG AGAGCACAGA TGGAGGAAAG 1700  
 GCCATTCTAA AAAATCACCT CGATCAAAAC CCCAGCTCC AGTGAACAC CACAGAGCCC 1760  
 TCAAGAACAT GCAAAGACCC CATAGAAGAC ATCAACTCCC CGGAGCACAT CCAGCGCCGG 1820  
 CTGTGCTCC AGCTCCCSAT CCTTCACCAC GCCTACCTCC CATCCATCGG AGGCGTGGAT 1880  
 GCGAGCTGGC TCAGCCCSCT TGTACGCCCT ACCGCCAGCC CTCGCCACAG ACACGTACCA 1940  
 CCTCTCTTCC GAGTCATGGT CTCGGGCTGT TAGGGGTGGG AGGCCTGGGG CCGGGGCTTC 2000  
 CCGGGGACAG CACCATGCTG GGCCAAGGCG CCTGCCACAG GCACACTGAC GCGGGGAGAG 2060  
 AGCTGGGCAC CATGCTGCCT CTCCAGACTG CTGGAATGGC GCTCAGGCAG AGCGGGACTC 2120  
 GGCACCGACC TCGAGCCTTA TCTGTGAAGG TCTTACTCTC ACAGAGGAGA GGAATGACAA 2180  
 TGACTTCTCC TTCTTGGCGT CTGCAACAA AGAGGAGTTG GGATGTCTGA AACTTGCAAW 2240  
 AACAAATCAA ACTCTAGACA AAGGAGAGAG GCCTCGGACT CCTGCTGTCC TCGCCAAGTG 2300  
 GCCAGAGCAA GGGCTCTGCA GAAATGGCCC TCGGCACCTC GCACACACGT CATCGGTGGT 2360  
 GCGCGCGCCC ACACACGAGC CTTTCTCCAC TCTGGGGGAG AAGGTGAGCA GGGCCACCAG 2420  
 GTCCCCAGCA GCTGGCCAG TCCACTTCAG ACTTCTGGA ACAAGGGAGT CAGCAGGAAG 2480  
 GGTAGCCGGG CTCTGCCCTG GGGCTGGGG AATCAGAGCA AGCAGCTCGG CATGCTCCCA 2540  
 GGGGACATCC ATCAGAAGAG AGATGTTTGT TACTCTCTGA TAAATGCCAC ACATTAACAC 2600  
 AATAACACCC GTTCCGGGGA CTGTGGGGAT TCAGGGCACC GCACTGCAGA CATGCTCTGC 2660  
 AGGATGCAAC AGACAGTCTG TCATGCACCT ACCGTGTCGG CCATGTTCTT GTGTTCAAAT 2720  
 TTAGGGTTCC CGGTAATAAG GGGAAACATC TGAGCTAATC ATGGAATGTC TGCTCCGAGA 2780  
 AAACACGATA AAGAAAGATT GTGCACTTTA ACCTCTCCCA TGGGGGCCCA AGGGGTGCTG 2840  
 GGATTCCTCC TCTCCCAACA CACTGACTTT GTTTTGGACC AAAGTGAATC AGGGGCATTTC 2900  
 TGCTAAAGT CATCTCTGTG TTCCTGAGCT AGAGCTGCTG GCCCAGGGCC CCTTGCTTC 2960  
 CAGAAGGTAG CCTCCAGTG CCTCTCCAGC CTAACCCGT GAGTGCCAC CTAGGGTGTG 3020  
 ACCTGAGGCG GTCARTCAGC AGGCTGGTTC CCATCCATCA CGACATACTT TCCTTGTAAT 3080  
 TGAACCCCAA AGAGCACTGG CCTGGGAGTC AGACCTGGAT TTGAGTCTC AGTCCCTTCA 3140  
 GACTCTCTCC GTGACCTGG GCAAGTCACC AGTGTCTCTC TGAGCCTCGG TTTCCCTCT 3200  
 GTAACAGACT GGGGTGAAA TAACACCCAT CCTGCCTACC TCACAGGGTC ACTCTGAGGA 3260  
 TGGACACTTG ATCTCATTCA TCCAGGAGAG CTTTGCACAG GGAAGCAGCT CTGACCTGTG 3320  
 AGGTATTAGT ACGACCATGA CCTTCAGCCC TCCCACCAGC TGGGGAGGGT GCGGTGCGGC 3380  
 CCAGAGAAAC TTCCTGCAAG GCGCTCTACC TAGGGACAGG AACAGCCAG TCAGAGGCAG 3440  
 GCTCAGGACT CTTCTGACAT TCCTTAATAG GTTCATTCTT TGCTAAAGGC CTGAATGGCA 3500  
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 AGCTTCAACA GGCAGGCAAT AATCTCTTCC CAGACCACTG CAGTCAGGAA CACACTGCTG 3620  
 TCAACCACCA GGTGTGACG AAAGGGCCCA CGGGAATCAC CATGCCAAC ATTTCAAAC 3680  
 ACCCTAGTTC ACGTAGCATA GCTCTTCTC CCCATCCCTC AAAGAGAGGT TACAGAGGT 3740  
 CTGTAGCTTT TAAGGGGGGA AAAATGTCAA CACATCACAG GTCAAGTTGA TGTCTCTC 3800  
 TGTTTAGGCA CCAAACTCC GTGTTGTAC TCACTGTGTG TGTTAACCAG TATTCTCTG 3860  
 CTTTTTCGAC TTCACCAAAA CCAAAATTCA TTTAAAGAC CGYATTAGTT TTTTCAAAG 3920  
 GGAAAGAGAT AATTAAGTGT ACATAATGTA TACAGACAAA ACAAACCTG TAGAAATATT 3980  
 ACTCCAGCAT AGCAGGAAAA AAAAAAAC AAAAAACAAA AAACAAAAGT ATTGGATTGT 4040  
 CGGAGGTGAG CGTGCGTCTG TAATCCCTTG TGAATCCGAA CTGTGCTCCG TCTCTAGGT 4100  
 TAACCCACAA GATACATCTC TGTCTACTGA TCCTGTAGGT TCACCCATTT TTTTAAAT 4160  
 TCTCTGCAAT TAACAAGCCC CACAAAGTGA TTCTGGCTAT CTACTGGTTC TGTCTTTTA 4220  
 TATGCAGCAA ACACACGGTC CATTTCCTAG AGGCTTCCGC CCAAGGGCAT CTTCAGTGA 4280  
 CGCTAGTGCG CACACACACA CACACACACA CACACACACA CACACACGAC 4340  
 TGCTTTCAAT TAGACCGGAA CTGGCAGAA CAAATGTAAA TGAGGAGTTT CTCTCGCCC 4400  
 ACTGCATGGT ATCGATTTTT AATAAATTGT TGCAAAATTG TTTTATGAA TAACAGGGG 4459

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 940 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: unknown  
 (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
1      5      10      15
Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu
20      25      30
Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
35      40      45
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
50      55      60
Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65      70      75      80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
85      90      95
Tyr Phe Leu Asp Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
100     105     110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
115     120     125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
130     135     140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145     150     155     160
Val Leu Ala Asp Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
165     170     175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Tyr
180     185     190
Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
195     200     205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
210     215     220
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225     230     235     240
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
245     250     255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met Tyr
260     265     270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser Trp
275     280     285
Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg Lys
290     295     300
Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305     310     315     320

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Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln  
 325 330 335  
 Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser Lys  
 340 345 350  
 Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu  
 355 360 365  
 Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile  
 370 375 380  
 Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu Asn  
 385 390 395 400  
 Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe  
 405 410 415  
 Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp  
 420 425 430  
 Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu  
 435 440 445  
 Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys  
 450 455 460  
 Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu  
 465 470 475 480  
 Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser  
 485 490 495  
 Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys  
 500 505 510  
 Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu  
 515 520 525  
 Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser  
 530 535 540  
 Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr  
 545 550 555 560  
 Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg  
 565 570 575  
 Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys  
 580 585 590  
 Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu  
 595 600 605  
 Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val  
 610 615 620  
 Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile  
 625 630 635 640  
 Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu  
 645 650 655  
 Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe  
 660 665 670  
 Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser  
 675 680 685  
 Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile  
 690 695 700  
 Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe  
 705 710 715 720  
 Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys  
 725 730 735  
 Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala  
 740 745 750

Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu  
 755 760 765  
 Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr  
 770 775 780  
 Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys  
 785 790 795 800  
 Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln  
 805 810 815  
 Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu  
 820 825 830  
 Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly  
 835 840 845  
 Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln  
 850 855 860  
 Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp  
 865 870 875 880  
 Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro  
 885 890 895  
 Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser  
 900 905 910  
 Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His  
 915 920 925  
 Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu  
 930 935 940

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 940 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: None

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro
1      5      10      15
Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Ser Leu Leu Leu
20     25     30
Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
35     40     45
Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
50     55     60
Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
65     70     75     80
Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
85     90     95
Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
100    105    110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
115    120    125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
130    135    140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145    150    155    160
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
165    170    175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
180    185    190
Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Xaa Gln Arg Phe Ser
195    200    205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
210    215    220
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225    230    235    240
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
245    250    255
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
260    265    270
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
275    280    285
Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
290    295    300
Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305    310    315    320
Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln

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      325              330              335
Phe Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
      340              345              350
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
      355              360              365
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
      370              375              380
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn
      385              390              395              400
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
      405              410              415
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
      420              425              430
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
      435              440              445
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
      450              455              460
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
      465              470              475              480
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
      485              490              495
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
      500              505              510
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
      515              520              525
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
      530              535              540
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
      545              550              555              560
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
      565              570              575
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
      580              585              590
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
      595              600              605
Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
      610              615              620
Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
      625              630              635              640
Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
      645              650              655
Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
      660              665              670
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
      675              680              685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
      690              695              700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
      705              710              715              720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
      725              730              735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
      740              745              750
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu

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755	760	765
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr		
770	775	780
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys		
785	790	795
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln		
805	810	815
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu		
820	825	830
Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly		
835	840	845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln		
850	855	860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp		
865	870	875
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro		
885	890	895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser		
900	905	910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His		
915	920	925
Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu		
930	935	940

**CLAIMS**

We claim:

- 5           1. An isolated and essentially pure DNA molecule which encodes a mammalian gb2 GABA<sub>B</sub> receptor.
2. The isolated and essentially pure DNA molecule of claim 1, wherein the DNA molecule is a cDNA molecule.
- 10           3. The isolated and essentially pure DNA molecule of claim 2, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a human gb2 GABA<sub>B</sub> receptor.
4. The isolated and essentially pure DNA molecule of claim 2, wherein the  
15 cDNA molecule has the sequence given by SEQ. ID NO.:1 or allelic variations thereof.
5. The isolated and essentially pure DNA molecule of claim 2, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a rat gb2 GABA<sub>B</sub> receptor.
- 20           6. The isolated and essentially pure DNA molecule of claim 2, wherein the cDNA molecule has the sequence given by SEQ. ID NO.:2 or allelic variations thereof.
7. An isolated mammalian GABA<sub>B</sub> receptor of subtype gb2.
- 25           8. The isolated mammalian GABA<sub>B</sub> receptor of claim 7, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a human gb2 GABA<sub>B</sub> receptor.
9. The isolated mammalian GABA<sub>B</sub> receptor of claim 7, wherein the human  
gb2 GABA<sub>B</sub> receptor has the sequence given by SEQ. ID NO.:3 or allelic variations  
30 thereof.

10. The isolated mammalian GABA<sub>B</sub> receptor of claim 7, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a rat gb2 GABA<sub>B</sub> receptor.
11. The isolated mammalian GABA<sub>B</sub> receptor of claim 7, wherein the rat gb2  
5 GABA<sub>B</sub> receptor has the sequence given by SEQ. ID NO.:4 or allelic variations thereof.
12. A recombinant DNA comprising a DNA molecule which encodes a mammalian gb2 GABA<sub>B</sub> receptor and which is operably linked to control nucleotide  
10 sequences capable of expressing the mammalian gb2 GABA<sub>B</sub> receptor in a compatible host.
13. The recombinant DNA of claim 12, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a human mammalian gb2 GABA<sub>B</sub> receptor.  
15
14. An isolated or pure clone from a mammalian tissue DNA library, wherein the clone contains DNA which encodes a mammalian gb2 GABA<sub>B</sub> receptor.
15. The isolated or pure clone of claim 14, wherein the clone is from a human  
20 tissue library and the DNA encodes a human gb2 GABA<sub>B</sub> receptor.
16. A sequencing vector for sequencing DNA which encodes a mammalian gb2 GABA<sub>B</sub> receptor, said vector comprising a sequencing vector and DNA, which encodes a mammalian gb2 GABA<sub>B</sub> receptor, inserted into the vector in proper  
25 orientation and correct reading frame for sequencing the DNA.
17. The sequencing vector of claim 16, wherein the DNA encodes a human gb2 GABA<sub>B</sub> receptor.

18. An expression vector for expressing DNA that codes for a mammalian gb2 GABA<sub>B</sub> receptor in a compatible host, said expression vector comprising a vector capable of transforming a procaryotic or eucaryotic cell and DNA which encodes a mammalian gb2 GABA<sub>B</sub> receptor inserted into the vector in proper orientation and  
5 correct reading frame for expression.

19. The expression vector of claim 18, wherein the DNA encodes a human gb2 GABA<sub>B</sub> receptor.

10 20. The expression vector of claim 19 wherein the cell is a mammalian cell.

21. A process for producing a mammalian gb2 GABA<sub>B</sub> receptor, said method comprising expressing the expression vector of claim 18 in the cell transformed by the vector.

15

22. A cell transformed with the expression vector of claim 18.

23. A mammalian cell line comprising a mammalian cell stably transfected with the expression vector of claim 18 and capable of continuous growth in a suitable  
20 culture medium.

24. The mammalian cell line of claim 23 wherein the mammalian cell is selected from the group consisting of a neuronal cell, a fibroblast cell, an epithelial-like cell, and a glial cell.

25

25. The mammalian cell line of claim 23 wherein the cells of the cell line express a human gb2 GABA<sub>B</sub> receptor having the amino acid sequence of SEQ. ID NO.:3 and allelic variants thereof.

30 26. A process for producing a human gb2 GABA<sub>B</sub> receptor, said process comprising culturing the mammalian cell line of claim 25 in a suitable culture medium.

27. A method of making a mammalian cell that expresses a mammalian gb2 GABA<sub>B</sub> receptor, said method comprising the steps of:

transfecting a mammalian cell that does not express the receptor with the expression vector of claim 182; and

5 recovering said transfected mammalian cell.

28. The method of claim 27, wherein the mammalian cell that does not express the receptor is taken from a cell line that is capable of continuous growth in a suitable culture medium and comprising the further step of cloning the transfected cell, thereby  
10 producing a cell line that is capable of continuous growth in a suitable culture medium, the cell line expressing mammalian gb2 GABA<sub>B</sub> receptor.

29. The method of claim 28, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a human gb2 GABA<sub>B</sub> receptor.

15

30. The mammalian cell produced by the method of claim 29

31. The mammalian cell of claim 30, wherein the mammalian gb2 GABA<sub>B</sub> receptor is a human gb2 GABA<sub>B</sub> receptor.

20

32. In a method for the pharmacological, physiological, functional, or other investigational analysis of a mammalian gb2 GABA<sub>B</sub> agonist or antagonist, the improvement comprising the use of an effective amount of the mammalian cell of claim 30 in the pharmacological, physiological, functional, or other investigational  
25 analysis.

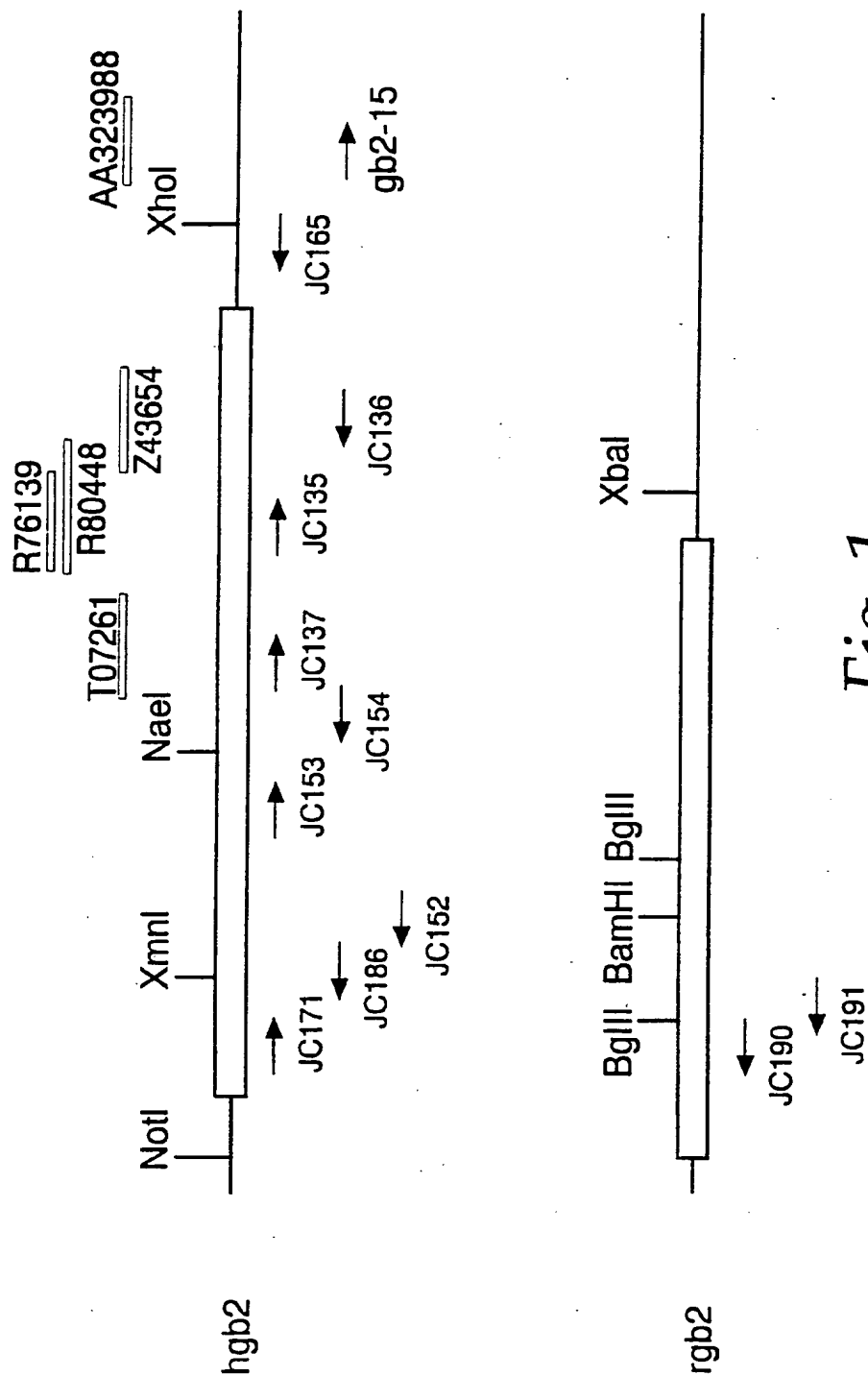
33. An *in vitro* method of determining the ability of a chemical to bind to a mammalian gb2 GABA<sub>B</sub> receptor, said method comprising the steps of:

contacting the chemical with a cell or part thereof from the mammalian cell of  
30 claim 23; and

determining the ability of said cell or part thereof to bind the chemical.

34. An in vitro method for evaluating a chemical to determine if it is a mammalian  $\text{GABA}_B$  receptor agonist, said method comprising the steps of:  
contacting the chemical with a cell or part thereof from the mammalian cell line of claim 23; and

5 measuring the effect of the chemical on a second messenger pathway of the cell or part thereof.



*Fig. 1*

```

hgb2  MASPRSSGQPGPPPPPPPPARLLLLLLPLLL-----
rgb2  MASPPSSGQPR-PPPPPPPPARLLLPLLLSLLL-----
rgb1a          MLLLLLVPLFLRPLGAGGAQTPNATSEGCQI | HPPWEGGI
rgb1b          MGPGGPCTPVGWPLPLLLVMAAGVAPVWA-----
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```

```

hgb2  -----
rgb2  -----
rgb1a RYRGLTRDQVKAINFLPVDYEIEYVCRGEREVVGPKVRKCLANGSWTDMDTPS
rgb1b -----

```

```

hgb2  -----
rgb2  -----
rgb1a RCVRI CSKSYLTLENGKVFLTGGDLPALD GARVEFRCDPDFHLVGSSRSVCSQ
rgb1b -----S-

```

```

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hgb2  PLAPG-AWGWARGAPRPPPS SPPLS IMGLMPLTKEVAKGS IGRGVLPAVELAI
rgb2  WLAPG-AWGWTRGAPRPPPS SPPLS IMGLMPLTKEVAKGS IGRGVLPAVELAI
rgb1a GQWSTP-KPHCQVNRTPHSERRAVYIGALFPMS- GGWPG--GQACQPAVEMAL
rgb1b HS-PHLPRPHPRVPPHPS SERRAVYIGALFPMS- GGWPG--GQACQPAVEMAL
          . . . . .

```

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          *
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rgb2  EQIRN-ESLLRPYFLDLRLYDTECDNAKGLKAFYDAIKYGNHLMVFGGVCPS
rgb1  EDVNSRRDILPDYELKLIHDSKCDPGQATKYLYELLYNDPIKIIIMPG-CSS
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```

```

hgb2  VTSIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAI LKLL
rgb2  VTSIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAI LKLL
rgb1  VSTLVAEARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVKLF
          . . . . .

```

```

hgb2  KHYQWKRVGTLTQDVQRFSEVRNDLTGVLYGEDIEISDTESFSNDPCTSVKKL
rgb2  KHFRWRRVGTLTQDCQRFSEVRNDLTGVLYGEDIEISDTESFSNDPCTSVKKL
rgb1  EKWGWKKIATIQQTTEVFTSLDDLEERVKEAGIEITFRQSFFSDPAVPVKNL
          . . . . .

```

```

hgb2  KGNDVRIILGQFDONMAAKVFCCAYEENMYGSKYQWIIPGWYELSWWEQVHTE
rgb2  KGNDVRIILGQFDONMAAKVFCCAFEESMFGSKYQWIIPGWYEPAWWEQVHVE
rgb1  KRQDARIIVGLFYETEARKVFCEVYKERLFGKKYVWFLIGWYADNWFK---TY
          . . . . .

```

```

          *
hgb2  ANSSRCLRKNNLLAAMEGYIGVDFEPLSSKQIKTISGKTPQQY-EREYNN-KRS
rgb2  ANSSRCLRRSLLAAMEGYIGVDFEPLSSKQIKTISGKTPQQF-EREYNS-KRS
rgb1  DPSINCTVEEMTEAVEGHITTEIVMLNPANTRSISNMTSQEFVEKLTKRLKRH
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hgb2  GVGPSKFH-G-YAYDGIWWIAKTLQRAMETLHASSRHQRIQDFNYTDHTLGR
rgb2  GVGPSKFH-G-YAYDGIWWIAKTLQRAMETLHASSRHQRIQDFNYTDHTLGKI
rgb1  PEETGGFQEAPLAYDAIWALALALNKTSGGGGRSGV---RLEDFNYYNQTITDQ
          . . . . .

```

Fig. 2A



\*  
 hgb2 I L N A M N E T N F F G V T G Q V V F R - N G E R M G T I K F T Q F Q D S R E V K V G E Y N A V A D T L E  
 rgb2 I L N A M N E T N F F G V T G Q V V F R - N G E R M G T I K F T Q F Q D S R E V K V G E Y N A V A D T L E  
 rgb1 I Y R A M N S S S F E G V S G H V V F D A S G S R M A W T L I E Q L Q G G S Y K K I G Y Y D S T K D D L -  
 . . . . .  
 \* TM1  
 hgb2 I I N D T I R F Q G S E P P K D K T I I L E Q L R K I S L P L Y S I L S A L T I L G M I M A S A F L F F N  
 rgb2 I I N D T I R F Q G S E P P K D K T I I L E Q L R K I S L P L Y S I L S A L T I L G M I M A S A F L F F N  
 rgb1 S W S K T D K W I G G S P P A D Q T L V I K T F R F L S Q K L F I S V S V L S S L G I V L A V V C L S F N  
 . . . . .  
 TM2 TM3  
 hgb2 I K N R N Q K L I K M S S P Y M N N L I I L G G M L S Y A S I F L F G L D G S F V S E K T F E T L C T V R  
 rgb2 I K N R N Q K L I K M S S P Y M N N L I I L G G M L S Y A S I F L F G L D G S F V S E K T F E T L C T V R  
 rgb1 I Y N S H V R Y I Q N S Q P N L N N L T A V G C S L A L A A V F P L G L D G Y H I G R S Q F P F V C Q A R  
 . . . . .  
 TM4  
 hgb2 T W I L T V G Y T T A F G A M F A K T W R V H A I F -- K N V K M - K K K I I K D Q K L L V I V G G M L L  
 rgb2 T W I L T V G Y T T A F G A M F A K T W R V H A I F -- K N V K M - K K K I I K D Q K L L V I V G G M L L  
 rgb1 L W L L G L G F S L G Y G S M F T K I W W V H T V F T K K E E K E W R K T L E P W K L Y A T V G L L V G  
 . . . . .  
 TM5  
 hgb2 I D L C I L I C W Q A V D P L R R T V E K Y S M E P D P A G R D I S I R P L L E H C E N T H M T I W L G I  
 rgb2 I D L C I L I C W Q A V D P L R R T V E R Y S M E P D P A G R D I S I R P L L E H C E N T H M T I W L G I  
 rgb1 M D V L T L A I W Q I V D P L H R T I E T F A K E E P K E D I D V S I L P Q L E H C S S K M N T W L G I  
 . . . . .  
 TM6  
 hgb2 V Y A Y K G L L M L F G C F L A W E T R N V S I P A L N D S K Y I G M S V Y N V G I M C I I G A A V S F L  
 rgb2 V Y A Y K G L L M L F G C F L A W E T R N V S I P A L N D S K Y I G M S V Y N V G I M C I I G A A V S F L  
 rgb1 F Y G Y K G L L L L L G I F L A Y E T K S V S T E K I N D H R A V G M A I Y N V A V L C L I T A P V T M I  
 . . . . .  
 TM7  
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 rgb2 T R D Q P N V Q F C I V A L V I I F C S T I T L C L V F V P K L I T L -- R T N P D A A T Q N - R R F - Q  
 rgb1 L S S Q Q D A A F A F A S L A I V F S S Y I T L V L F V P K M R R L I T R G E W Q S E T Q D T M K T G S  
 . . . . .  
 hgb2 F T Q N Q K K E D S K - T S T S V T S V N Q A S T S R L E G L Q S E N H R L R M K I T E L D K D L E E V T  
 rgb2 F T Q N Q K K E D S K - T S T S V T S V N Q A S T S R L E G L Q S E N H R L R M K I T E L D K D L E E V T  
 rgb1 S T N N N E E E K S R L L E K E N R E L E K I I A E K E E R V - S E L R H Q L Q S R Q Q L R S R R H P P T  
 . . . . .  
 hgb2 M Q L Q D T P E K T T Y I K Q N H Y Q E L N D I L N L G N F T E S T D G G K A I L K N H L D Q N P Q L Q W  
 rgb2 M Q L Q D T P E K T T Y I K Q N H Y Q E L N D I L S L G N F T E S T D G G K A I L K N H L D Q N P Q L Q W  
 rgb1 P P D P S G G L P R G P S E P P D R L S C D G S R V H L L Y K  
 . . . . .  
 hgb2(a) N T T E P S R T C K D P I E D I N S P E H I Q R R L S L Q L P I L H H A Y L P S I G G V D A S C V S  
 hgb2(b) N T T E P S R T C K D P I E D I N S P E H I Q R R L S L Q L P I L H H -----  
 hgb2(c) N T T E P S R T C K D P I E D I N S P E H I Q R R L S L Q L P I L H H A Y L P S I G G V D A S C V S  
 hgb2 N T T E P S R T C K D P I E D I N S P E H I Q R R L S L Q L P I L H H A Y L P S I G G V D A S C V S

Fig. 2B

hgb2(a) PCVSPTASPRHRHVPPSFRVMVSGL  
hgb2(b) -----RHVPPSFRVMVSGL  
hgb2(c) PCVSPTASPRHRTTLGRGVCCRNTVGSGCGEAGHHGWPLRTTRMALRWTG  
rgb2 PCVSPTASPRHRHVPPSFRVMVSGL  
hgb2(c) RGRGRLGT

*Fig. 2C*

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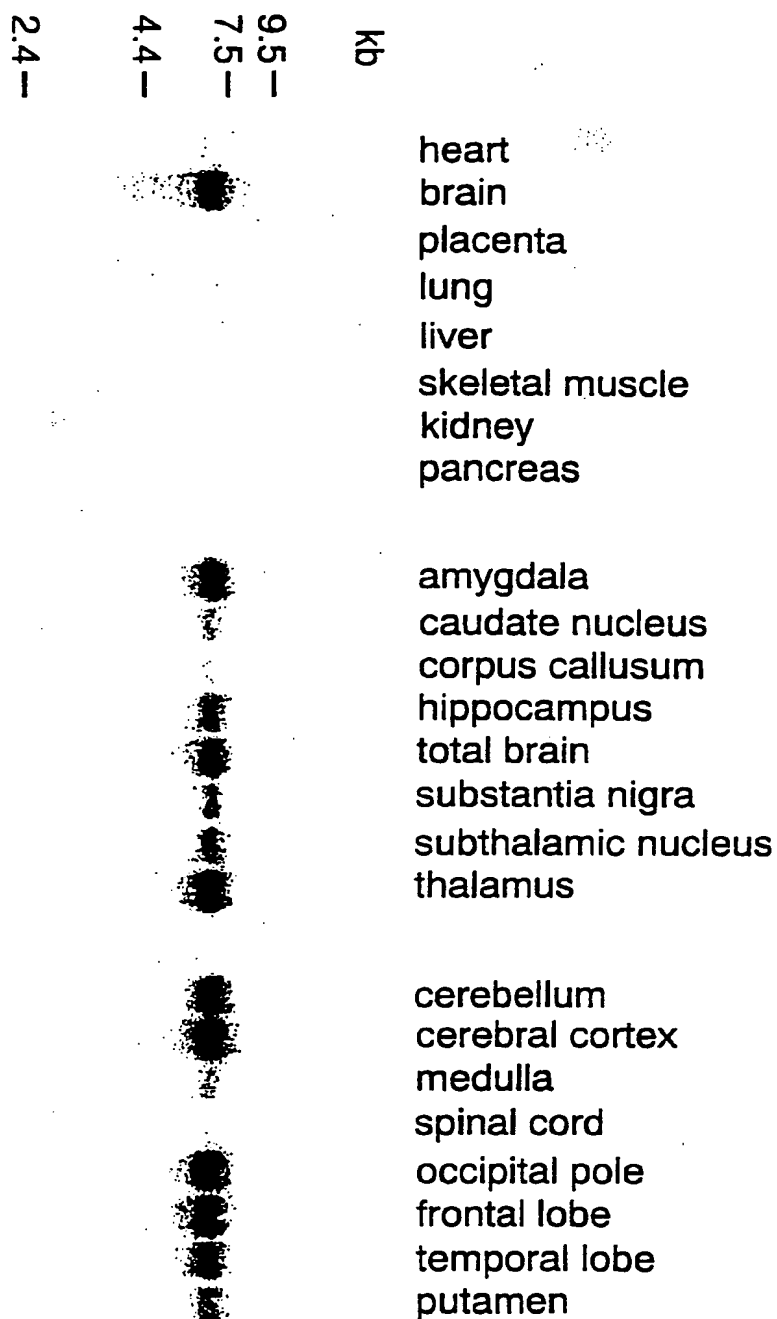


Fig. 3

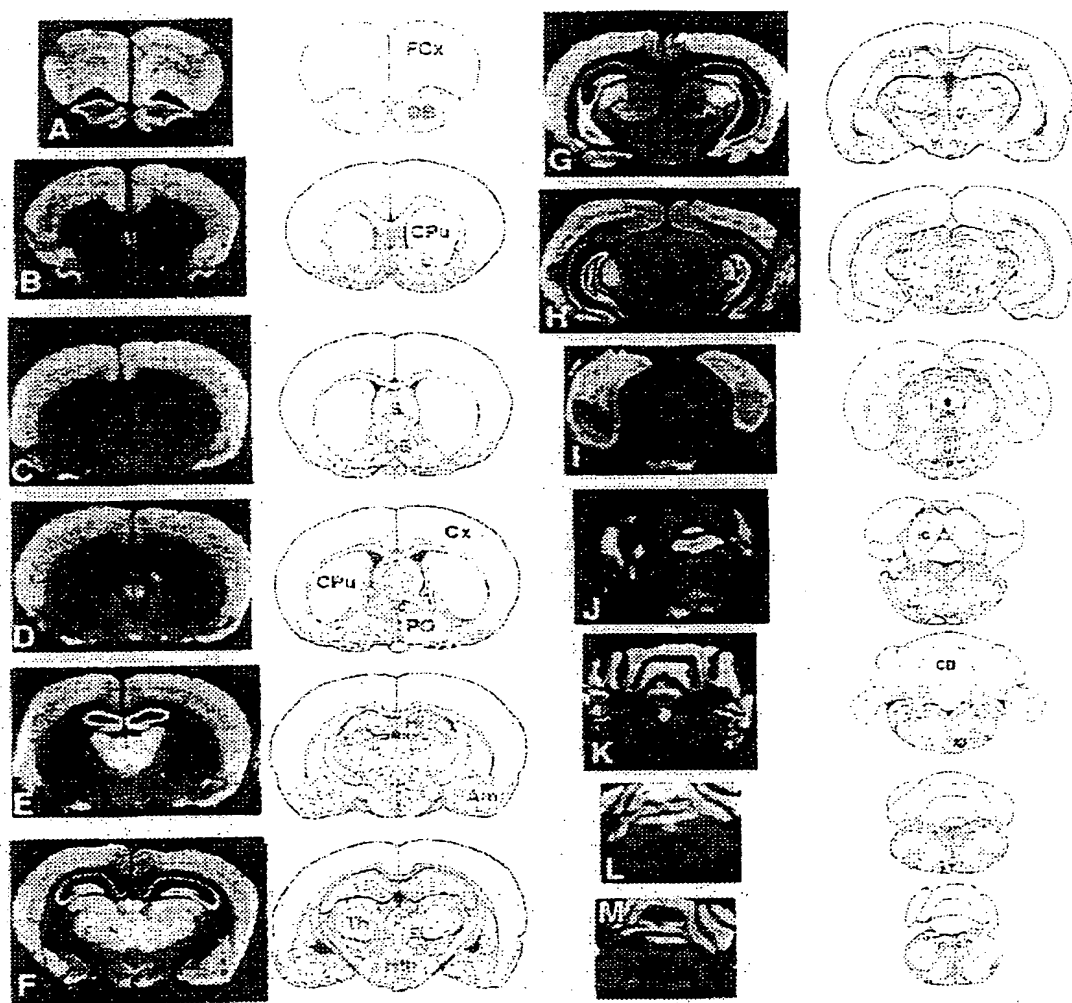
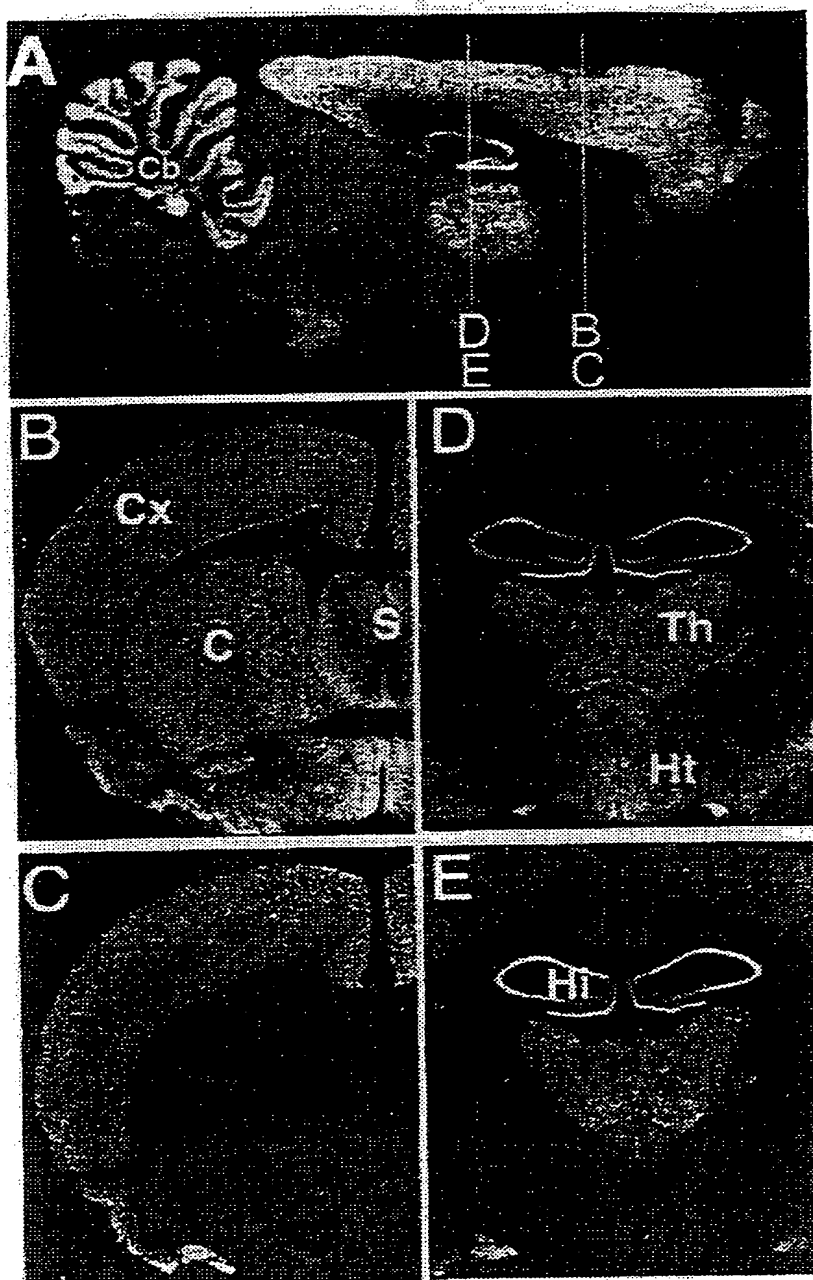


FIGURE 4

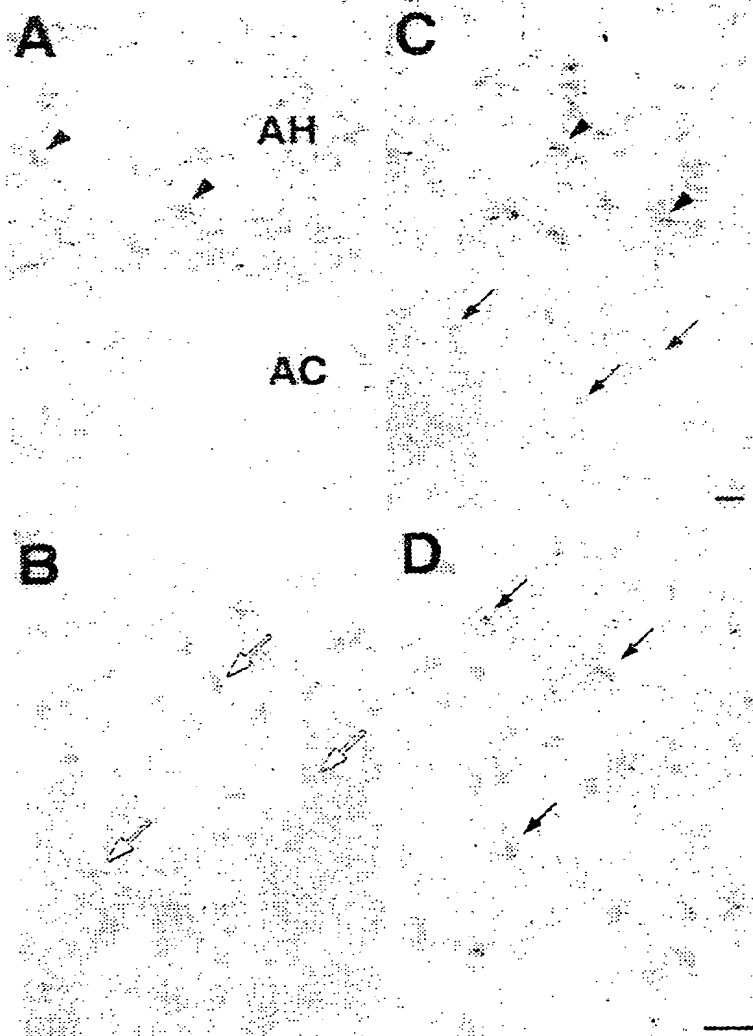
SUBSTITUTE SHEET (RULE 26)

FIGURE 5

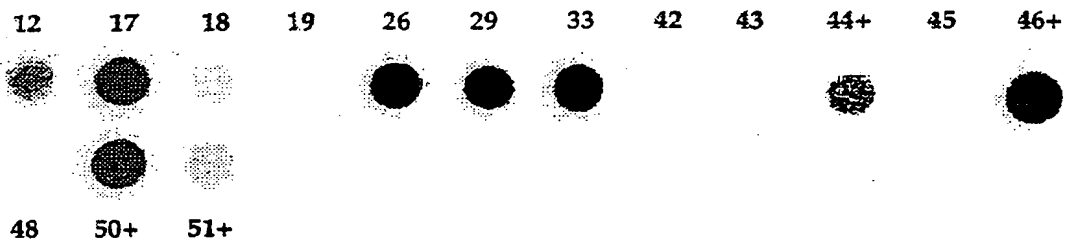
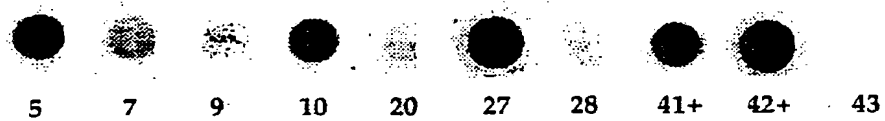


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FIGURE 6



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**FIGURE 7****A****HEK-293 rgb1a stable cell lines****B.****HEK-293 hgb2 stable cell lines**

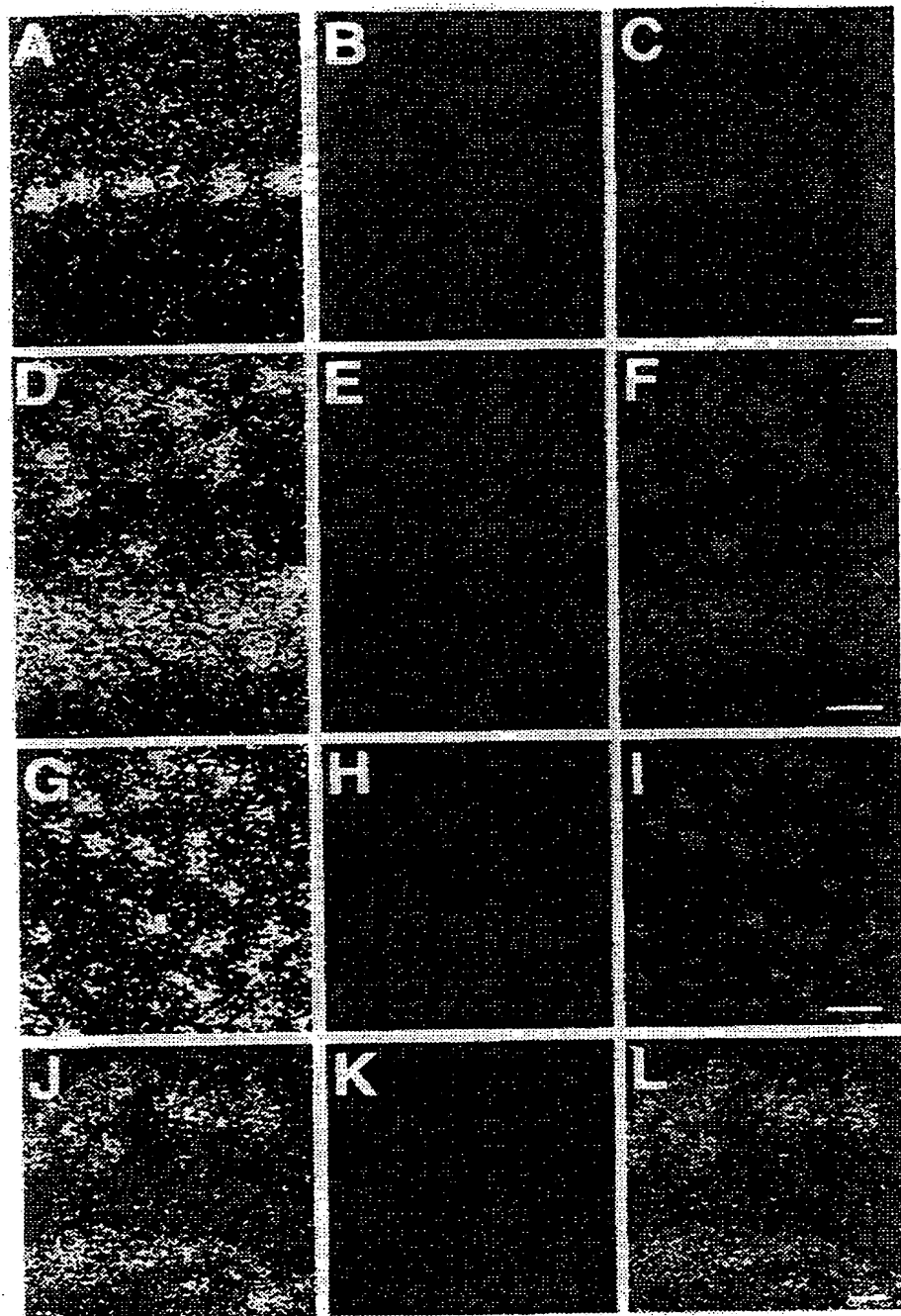
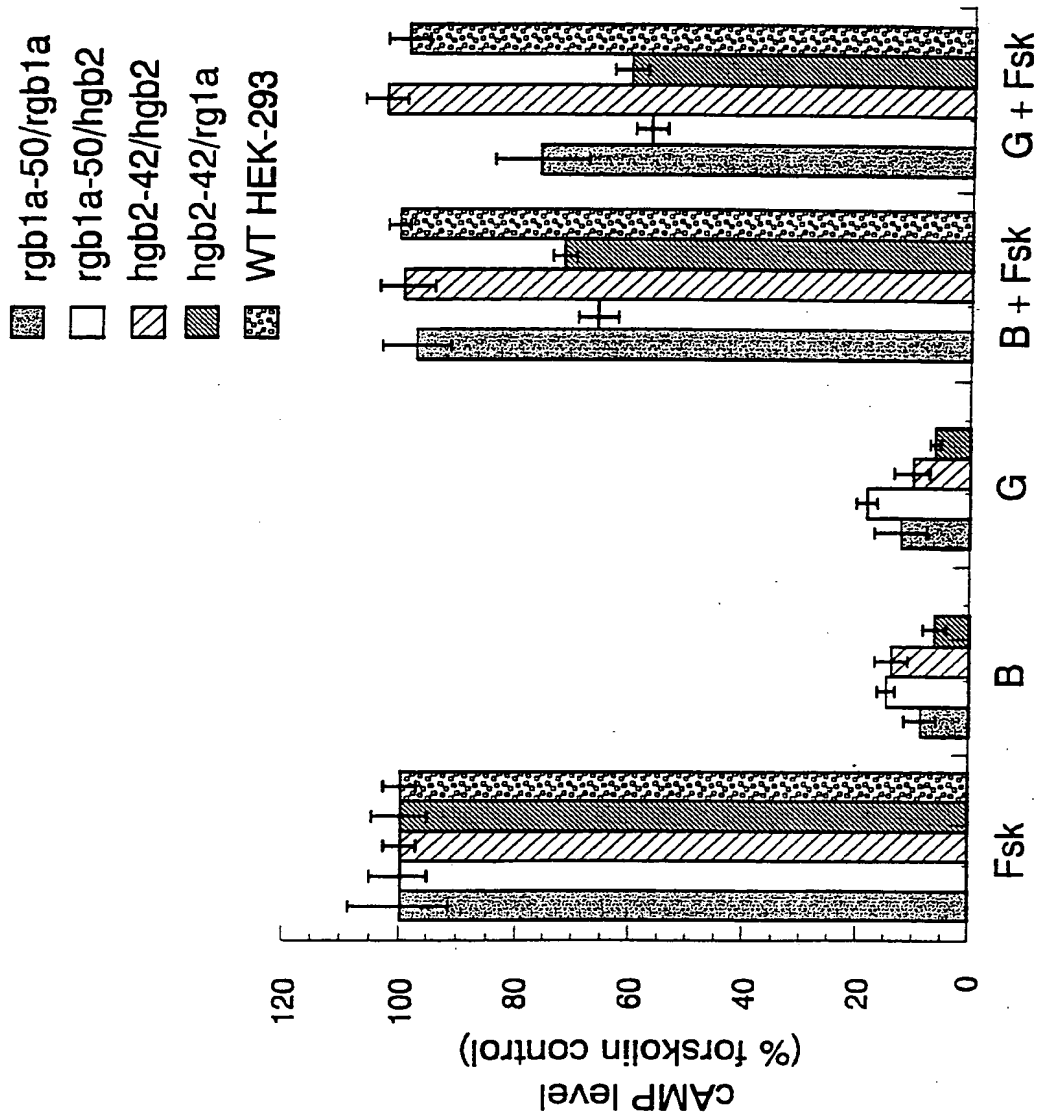


FIGURE 8  
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Fig. 9



## SEQUENCE LISTING

<110> United States of America, represented by Sec. HHS  
Clark, Janet  
Bonner, Tom I.

<120> Mammalian gb2 GABAB Receptors

<130> 65879

<150> 60/087,274

<151> 1998-05-29

<160> 30

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<210> 1

<211> 5786

<212> DNA

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35

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36

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43

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<213> Homo sapiens

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&lt;210&gt; 29

&lt;211&gt; 914

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

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-12-

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Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
675      680      685
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
690      695      700
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705      710      715      720
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
725      730      735
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
740      745      750
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
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Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
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<212> PRT
<213> Homo sapiens

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35      40      45
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
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Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65      70      75      80
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
85      90      95
Tyr Phe Leu Asp Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
100      105      110
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
115      120      125
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
130      135      140
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145      150      155      160
Val Leu Ala Asp Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
165      170      175
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Tyr
180      185      190
Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
195      200      205
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
210      215      220

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 Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser  
 530 535 540  
 Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr  
 545 550 555 560  
 Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg  
 565 570 575  
 Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys  
 580 585 590  
 Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu  
 595 600 605  
 Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val  
 610 615 620  
 Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile  
 625 630 635 640  
 Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu  
 645 650 655  
 Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe  
 660 665 670  
 Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser  
 675 680 685  
 Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile  
 690 695 700  
 Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe  
 705 710 715 720  
 Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys  
 725 730 735  
 Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala  
 740 745 750  
 Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu  
 755 760 765  
 Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr  
 770 775 780  
 Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys  
 785 790 795 800

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Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
      805      810
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
      820      825      830
Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
      835      840      845
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
      850      855      860
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
      865      870      875      880
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
      885      890      895
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
      900      905      910
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg Thr
      915      920      925
Thr Leu Gly Arg Gly Val Cys Cys Arg Asn Thr Val Gly Ser Gly Cys
      930      935      940
Gly Glu Ala Gly His His Gly Trp Pro Leu Arg Thr Thr Arg Met Ala
      945      950      955      960
Leu Arg Trp Thr Gly
      965

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/11869

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 15/10, 15/12, 5/10; C07K 14/705, 16/28; G01N 33/53

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.21, 69.1, 320.1, 325, 252.3, 254.11; 530/350, 387. 9, 388.22; 536/23.1, 23. 5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, APS, GenBank

search terms: j. clark, t bonner, gabab, gb2, r2

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor mRNA, complete cds.'. 08 October 1998, Accession Number AF056085.	1-4
X, P	Database GenBank, CLARK et al. 'gb2, A second GABA-B receptor. Rattus norvegicus GABA-B receptor gb2 mRNA, complete cds.'. 15 October 1998, Accession Number AF058795.	1, 2, 5
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor splice variant 1 mRNA, partial cds.'. 08 October 1998, Accession Number AF095723.	1-4

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

02 SEPTEMBER 1999

Date of mailing of the international search report

18 OCT 1999

Name and mailing address of the ISA/US  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/11869

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Database GenBank, CLARK et al. 'gb2, a second GABA-B receptor. Homo sapiens GABA-B receptor splice variant 2 mRNA, parital cds.'. 08 October 1998, Accession Number AF095724.	1-4
X, P	Database GenBank, LIU et al. 'Cloning and characterization of a novel human GABA-B receptor subtype with high affinity for GABA and low affinity for baclofen'. 17 May 1999, Accession Number AF095784.	1-3
X, P	Database GenBank, BOROWSKY et al. 'Direct Submission. Homo sapiens GABA-B receptor 2 mRNA, complete cds.' 07 January 1999, Accession Number AF074483.	1-3
X, P	WHITE et al. Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature. 24 April 1999. Vol. 396, No. 6712, pages 679-682, especially Figure 1.	1-4, 6-11, 13-29
X, P	JONE et al. GABAB receptors function as a heterodimeric assembly of the subunits GABABR1 and GABABR2. Nature. 17 December 1998. Vol. 396, pages 674-678, especially Figure 1.	1,2,5,6, 9,12-14,17- 20, 23, 26, 29
X, P	KAUPMANN et al. GABAB-receptor subtypes assemble into functional heteromeric complexes. Nature. 17 December 1998. Vol. 396, pages 683-687.	1, 2, 5, 6, 9, 12- 14, 17-20, 23, 26, 29, 30
X, P	KUNER et al. Role of heteromer formation in GABAB receptor function. Science. 01 January 1999. Vol. 283, pages 74-77.	1, 2, 5, 6, 9, 12- 14, 17-20, 23, 26, 29

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INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/11869

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

435/7.21, 69.1, 320.1, 325, 252.3, 254.11; 530/350, 387. 9, 388.22; 536/23.1, 23. 5